

nature

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For the city and for the world

PROFESSOR Daniel Bell of Harvard and sabbatically at the London School of Economics was lecturing last week at the United States Embassy in London on social institutions in post-industrial society. It was Daniel Bell, of course, who all but invented the term 'post-industrial society' and who has written extensively on the subject of a world in which knowledge is the major resource. He contends that the scale of many of our institutions (in tune, perhaps, with earlier days in which the major resource was land or machinery) is not necessarily in very good register with the sort of demands that will be made in a post-industrial society. An organisation that is co-extensive with a nation or a state may be too large for some purposes, too small for others.

In Professor Bell's *The Coming of Post-Industrial Society* (Heinemann, 1974) it was the scientists and the somewhat quaintly labelled 'research men' who would become the dominant figures where the landowners, the military and, more recently, the businessmen had once been. It follows, then, that the scale of organisations which sponsor, stimulate, monitor or control science is as much in question as in the scale of other social and economic institutions. Is there such a thing as a right size for administering science? This question has been raised recently in Scotland, as Martin Goldman remarked in last week's *Nature* (page 208), but it is not simply one of being ineffective if below some supposed critical mass; on the contrary it may be that the United States, Britain, Scotland, even the Greater London Council are too big for some purposes.

At this small end of the spectrum the local problem with a scientific content has frequently been sadly neglected. Polytechnics do what they can within the heavy teaching load, but universities rarely have much sense of springing from the local community, the academic staff are unlikely to be of local origin and the administration is likely to be more worried by the changing scene in the capital city than in the local town hall. As a result, peculiarly local questions of health, environment, resources and stimulation of enterprise are frequently passed over; besides, scientists are

rewarded more by fame than fortune, and you don't get many people receiving wide acclaim for solving a problem at the local quarry or sewage works. There seems to be a whole range of scientific questions on this small scale needing small institutions to initiate them.

But, some will argue, if we are weak at the small scale, we are at least capable of operating, when needed, at the supranational level. Look at CERN IPOD, ESA, ILL; all institutions which work quite well on an international basis. Certainly scientists, when the financial pressure is on, find little difficulty in sinking national differences. This is hardly surprising since scientific knowledge, of necessity, transcends national frontiers. But what is surprising is that this great international spirit has still made precious little impact at the science policy level. One example would be in the field of medical research. Every developed country must be seen to be funding a coherent programme to fight cancer just as it must have its own airline. The health problems of the developing world, arguably much more pressing than those of the developed world, receive a few per cent of our attention and yet we know full well that developing countries are unlikely to have the resources to support adequate research themselves.

There have, of course, been tentative efforts towards regional science policies and talk by individuals of something resembling a global science policy, but as yet the power and pride of nations has not allowed much progress along a direction which is going to cut across a lot of autonomies and cosy relationships. Maybe pressure in this direction will eventually come from the financial problems of supporting extensive national scientific programmes. One would hope, however, that pressure might come from scientists themselves for international decision-making and international programmes over a very wide range of disciplines.

Daniel Bell's challenge is this: to ensure, as knowledge becomes the major resource, that it is sought in the areas where there is greatest need and is distributed fairly and rapidly. We would do well to ponder whether our present institutions are best organised for this task. □



A fair exchange?

Philip Hanson assesses the balance in the East-West transfer of technology

THE technological relations between the West and the Soviet bloc are curious. The United States and the Soviet Union spend huge sums on military R&D, primarily to maintain their abilities to blow one another to pieces. Yet at the same time Western businessmen are eagerly selling know-how and high-technology products to the USSR and Eastern Europe; and there is also a certain commercial flow of technology—of more modest dimensions—from East to West.

These transactions, of course, are in principle restricted by strategic embargoes to non-military purposes; yet the technologies transferred quite often originate from military R&D. In any case the civilian-military distinction is somewhat artificial: in the long run any new technology that makes the civilian economy more productive makes it easier to enlarge the resources allocated to the military. And even if there were no military considerations, economic competition between communist and capitalist countries is part of their general competition for power and influence, and so much technical assistance among competitors—even at a price—is at first sight rather odd. Altogether it is not surprising that East-West technology transfer is politically controversial.

The recent growth of Soviet-US trade, in particular, has prompted economists to look more closely than before at East-West technological relations. They have focused, naturally enough on commercial transactions. Obviously there are many ways in which knowledge about new products and processes travels from West to East without any commercial exchange, but these are not discussed much because most of them are not amenable to control by governments. The screening of Western technical literature by Soviet specialists, for example, and the gleaning of information from visits and conferences are beyond the influence of all but the most provocative action by governments.

East-West trade, however, provides a number of channels of technology transfer: notably, the sale of products embodying new technology; turnkey projects; licencing; know-how sales; and technical assistance and training. Some of these transactions simply provide cost-saving machinery and other products (for example wide-diameter pipe for gas pipelines) with-

out transmitting the know-how to make the product in question. Other transactions, however, such as licence deals with technical assistance, or joint design work, may transfer such capabilities.

An interesting example is the Pullman-Swindell contribution to the huge foundry complex at the Soviet Kama River lorry works. Over a period of three years a total of more than 150 Soviet engineers worked with Pullman-Swindell on the detailed design and equipment ordering. The knowledge they acquired must have been considerable. How far it would enable the Russians to repeat the exercise for themselves probably depends on the extent to which these particular engineers can be kept together as a design team.

Most Western governments subsidise export credit as they compete against one another to promote exports and they thus ease the terms on which technology is transferred. Is this really in the interests of the West as a whole? Would it be wiser not to encourage such transfers, or even to restrict them? What is or is not wise in this context is largely a matter of political judgment: in other words, nobody really knows. The economics of East-West technology transfer, however, are more susceptible to measurement, and a good deal of measurement has accordingly been going on. The aim is to quantify the economic costs and benefits to Western and Soviet-bloc countries of transactions by which technology is transferred.

The starting point is the well established fact that in most fields Soviet and East European civilian production lags technically well behind that of the West even though these countries maintain large R&D establishments and are strong in many areas of basic research. Heavy military priorities, semi-isolation from Western technology and the lack of competitive pressures and incentives, however, have resulted in a generally poor performance in the creation and diffusion of new technologies. They therefore stand to gain a good deal from the acquisition of Western know-how.

Clearly Western firms also gain from selling this know-how. A recent survey of 168 US firms selling to the USSR found—in contrast to what some economists had predicted—that about three-quarters of them considered their activities to be profitable. The position

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Novosti

West-East: Fiat's USSR showroom

of West European firms, who have had more experience of dealing with the Russians, is probably more favourable.

The transfer that is going on, whether by means of product or of know-how sales, is predominantly of proven technology. In electronics, for example, Western firms quite often sell their latest products or the know-how to manufacture their less advanced products, but they seldom transfer the know-how to manufacture their latest products—let alone those currently under development. There is also, in the case of the Soviet Union, evidence that absorption and utilisation of imported technology is slow by Western standards. As a result, there is so far no very obvious narrowing of East-West technology gaps as a result of transfer.

Western firms, in other words, have not gone into Eastern markets in order to establish Eastern competitors for themselves. It is rather odd that anyone should ever have expected them to. Since they could not set up wholly- or majority-owned subsidiaries in any Comecon country, they have mostly been careful to hand over technology in measured doses: in products, in licence deals subject to market restrictions, and so on. More than a hundred companies, mostly big multinationals, have signed technological cooperation agreements with the Soviet State Committee for Science and Technology, but exchanges of information under many of these agreements have been minimal.

Production benefit

There is a good deal more, however, to the economics of East-West technology transfer than the question of its commercial profitability. Much of the debate in the West concerns the im-

pact of imported Western technology on Soviet production, and this raises some quite different questions. To begin with, there is the question whether imports of Western technology are really of more than marginal importance to the Soviet economy. The USSR has traditionally been highly self-sufficient. Only a very small percentage of its consumption and investment needs are met by imports. Less than half of these imports, moreover, have been from the West, and of this, capital goods—the main vehicle of international technology transfer—have generally comprised about a third. This means that in 1976, for example, imported Western machinery was almost certainly not more than 6% by value of total machinery installed. (The large differences between Soviet domestic prices and the prices of imports make precise comparison impossible.)

At first sight, this suggests that imported Western technology cannot have more than a very minor effect on Soviet output. If the growth of production depends on the growth of the labour force, the growth of the capital stock and technical change, then the technical change embodied in a very tiny part of the increment in the capital stock must surely make only a tiny contribution to that growth.

This need not follow, however, if the technology embodied in the imported machinery has significant indirect, as well as direct, effects. The direct effect of the imported technology is the extra output attributable to it, over and above what would have been produced with the same resources of capital and labour with the technologies already in use inside the country. But this same technology will also have in-

direct effects insofar as it is successfully diffused (for example by building Soviet machinery to the same design) and insofar as it enables improved producer goods (such as compound fertilisers) to be made which themselves raise productivity in other lines of production.

Soviet machinery and know-how imports have been strongly concentrated in certain fields: the main recipients have been the chemical industry, the motor industry, computerisation, shipping, and oil and gas pipeline transport. The planners have identified areas where there was a considerable technology lag: in other words, areas where the direct and indirect impact of technology would be considerable. The production of fertilisers and pesticides is an example: the backwardness of both the Soviet chemical industry and Soviet agriculture promised high returns to hard-currency spending on imported technology.

Some recent estimates suggest that the returns have indeed been high. One tentative estimate for the mineral fertiliser industry is that by 1975 the technology embodied in about \$2,000 million worth of installed Western equipment was yielding, directly and indirectly, some \$4,000 million worth of extra agricultural output per annum, over and above what the same resources would have produced in the absence of the imported technology. Part of the gain came from the diffusion of Western phosphoric acid and compound fertiliser technology to Soviet built plants.

A global estimate has been derived from an econometric model of the Soviet economy. The conclusion in this case was that, of an overall increase in Soviet industrial output of 32.1% between 1968 and 1973, 2.5% could be attributed to an acceleration of the import of Western machinery over that period. The acceleration was such that the stock of installed Western machinery was about a fifth higher in 1973 than it would have been if the rate of growth of imports had remained unchanged. Like the estimate for the mineral fertiliser industry, this entails a very high rate of return.

On the other side of the scale, the contribution of East-West technology transfer to Western GNP is very small indeed. The Soviet Union has probably made slightly over 200 licence sales to Western firms in recent years, mostly unaccompanied by machinery, and often requiring development and adaptation in the West. And the employment associated with Western capital-goods sales to the Soviet bloc is probably less than 0.5% of total em-

ployment in most OECD countries.

According to the conventional wisdom, Western governments should not worry unduly about all this because the Soviet economic system is relatively poor at using and diffusing new technology, and this fundamental lack of technological dynamism will keep the level of Soviet civilian technology generally below that of the West, even if the import of Western technology and know-how continues to grow strongly.

One way street

This may well be so. But if the two estimates just described are anywhere near correct they put this conventional wisdom in a somewhat changed perspective. They suggest that, while the Soviet economic system may be slower than Western economies in using and diffusing new technologies, it nonetheless does get a fair mileage out of imported technology. Where there is a particularly large backlog to be exploited, the impact on production can be dramatic.

This means that those who see East-West technology transfer as a 'one-way street', in which the benefits go mainly from West to East, have a serious case. It may be true that imported Western technology is not enabling the USSR to catch up and overtake the West in areas of advanced technology, but it is probably making a significant contribution to Soviet productivity levels, and thus reducing the pressure on Soviet policymakers to divert resources from military to civilian purposes.

Similarly, the firms selling the technology are no doubt doing so on commercially acceptable terms. On the other hand they are generally competing with one another to extract extra revenue from products and processes whose development costs have already been written off; they are therefore unlikely to charge prices that would come close to reflecting either the cost to the Soviet-bloc purchaser of replicating the development work for himself or his gains in output from using the technology.

The balance of economic advantage, then, in East-West trade in machinery and know-how seems to be strongly in the Soviet bloc's favour. From a conventional Western point of view this is not a welcome conclusion. Further evidence might possibly alter it, or it might be made more palatable by clear indications that the growth of East-West trade was linked with political advantage for the West. If not, a rather awkward question arises: is there anything much that Western governments can do about it? □

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USA

After the carrot, the stick

Colin Norman reports from Washington on the moves to introduce legislation to control recombinant DNA research in the United States

JUST two years ago, at the now famous Asilomar Conference, a group of scientists was discussing a set of proposed guidelines for experiments involving the powerful new recombinant DNA genetic engineering technology. Joshua Lederberg, a Nobel Prizewinner from Stanford who had been sounding ominous warnings about threats to free scientific inquiry, gloomily told his colleagues that there was "a graver likelihood of (these proposals) crystallising into legislation than some of us would like to think". That likelihood is now a virtual certainty.

Last week, the health subcommittee of the House of Representatives held three days of intense public hearings to examine a number of bills, including one proposed by the subcommittee chairman Paul Rogers, which seek to regulate all recombinant DNA experiments in the United States. Next week, the House subcommittee on Science, Research and Technology will begin its own set of hearings. On April 6, the Senate health subcommittee, chaired by Senator Edward Kennedy, will hold a hearing to discuss various

legislative proposals already introduced into the Senate, and Kennedy will probably subsequently offer a bill of his own. And legislation which will soon be proposed by the Carter Administration is now taking shape (see below).

Thus, what Lederberg correctly perceived as an inexorable march toward federal legislation is now entering the home stretch. It will take several weeks, perhaps months, for a final bill to emerge from Congress, however, and the debate is likely to turn on a relatively small number of issues.

First, it should be noted that Congress is unlikely to try to rewrite the guidelines issued last year by the National Institutes of Health (NIH). Though it is possible that some legislators will offer proposals to ban the research entirely—Representative Richard Ottinger said at last week's hearings, for example, that he is considering offering a bill declaring a moratorium and calling for an international meeting to develop a better understanding of the hazards—it is unlikely that such a move would succeed.

The most probable outcome is that Congress will direct the Secretary of Health, Education and Welfare (HEW) to develop regulations based on the NIH guidelines. Both the Rogers bill and the Administration's draft bill take that approach, and Kennedy said

in a statement last week that "we must assure sufficient and meaningful involvement of laymen and the general public in the formulation of regulations by the Secretary", thus indicating that he is likely to support that approach as well.

A second point to note is that the final regulations will apply equally to research supported by public and private funds, and that the legislation will establish a mechanism by which the controls can be enforced. Those two elements are missing from the NIH guidelines, and the need to extend and enforce the guidelines is largely responsible for the momentum now behind the development of federal legislation.

The coming Congressional debate is thus likely to centre on four areas:

- **The extent to which the federal regulations will override state and local controls.** Many scientists who were once opposed to federal legislation now support it because several state and local governments, led by the City of Cambridge, are developing their own controls on recombinant DNA experiments, thus raising the possibility that a patchwork of regulations of varying strictness will be adopted throughout the United States. Most scientists are consequently hoping that federal regulations will specifically prohibit local governments from setting their own controls on the research.

The Administration's draft bill would

A committee of government officials, representing 16 federal agencies, last week urged the Carter Administration to draft legislation to control all recombinant DNA research in the United States, and the committee sketched out the chief elements which it believes should be included in such a bill. As soon as the committee's report landed on his desk, the Secretary of Health, Education and Welfare (HEW), Joseph A. Califano, announced that his department would promptly transcribe the recommendations into formal legislation, noting that "such a measure is necessary not just to safeguard the public but also to assure the continuation of basic research in this vital scientific area".

The bill is expected to be ready early in April, when it will be submitted to Congress with an endorsement from the White House. It is likely to be particularly influential as Congress grapples during the next few weeks with the complex problem of how recombinant DNA research should be regulated.

The inter-agency committee, chaired by Donald Fredrickson, Director of the National Institutes of Health (NIH), began last November to seek ways to extend NIH's own recombinant DNA guidelines to cover experiments supported by other government agencies and by private industry. Though other agencies have since adopted the NIH

guidelines, the committee decided that new legislation is needed to ensure compliance by everybody conducting recombinant DNA experiments in the USA.

The committee consequently recommended that the Administration's bill should contain the following provisions. The Secretary of HEW should issue regulations based on the NIH guidelines, "with such clarifications and modifications as the Secretary determines to be necessary". All recombinant DNA experiments should be conducted at facilities licensed by HEW, the licence being issued only when the Secretary is satisfied that the facility will be operated in accordance with the regulations. Researchers would have to register their experiments with HEW. The committee suggests, however, that the Secretary could exempt from licensing and registration requirements "categories of activities which he determines pose no unreasonable risk to health or the environment".

To ensure that the regulations are being followed, HEW would have the authority to inspect facilities, conduct environmental and health monitoring, and require that reports and records be kept. The Secretary would have power to halt experiments if necessary, and violations of the regulations could incur loss of a facility's licence. In the event of injury to people or the environment, actions

and damages would fall under state and local laws.

The committee suggests that all reports submitted to HEW by experimenters and officials in research facilities should be made available to the public on request, except for information likely to cause loss of proprietary rights. Before information is released, researchers would be able to identify material which they believe should be kept confidential, but the final decision on disclosure should rest with the Secretary of HEW.

In what may turn out to be one of its most controversial recommendations, the committee suggests that federal legislation should specifically pre-empt all state and local regulations regarding recombinant DNA research. The only exception would be if a state passes a law imposing requirements identical to those contained in the federal legislation, the Secretary of HEW could enter into an agreement with the state to carry out the inspection, monitoring and enforcement responsibilities. In other words, such a clause would rule out the setting of state and local controls of varying stringency.

Finally, the committee recommends that the legislation should contain provisions protecting the rights of employees who blow the whistle on their superiors, and it also suggests that the legislation should lapse after five years unless further action is taken by the Congress.

indeed pre-empt local moves, though it would allow the Secretary of HEW to enter into agreements with state governments under which local agencies would enforce the federal regulations. The Rogers bill, however, would allow the Secretary of HEW to approve state regulations which are at least as strict as the federal controls, and Kennedy said last week that "we must carefully assess the wisdom of totally pre-empting state and local laws regulating the recombinant DNA research and applications given our concern to assure the involvement of the general public in these issues".

The crux of the issue is how the federal government can ensure the adoption of uniform regulations without throttling public debate and without cutting off legitimate community interest in what universities are up to.

● **The nature of registration and licencing schemes.** It is likely that the final version of the legislation will establish a mechanism through which researchers or research facilities would be licenced and research projects would be registered with the federal government. There is a significant difference in approach between the Administration's draft bill and the Rogers bill, however, and it is not clear which approach will prevail.

The Administration's draft bill, in short, would require that facilities housing recombinant DNA experiments be licenced by HEW and that individual research projects would simply have to be registered with HEW. The Rogers bill, on the other hand, would require researchers themselves to obtain licences from HEW before embarking on any recombinant DNA project, and the licence application would specify the nature of the project and the safeguards to be employed. The difference between the two approaches represents a very significant difference in the focus of control and the complexity of the licencing process. Kennedy has so far made no statement on that aspect of the legislation.

● **The extent to which proprietary information can be protected from public disclosure.** There is a clash of interests between the need on the one hand to provide full public information on recombinant DNA experiments and the need on the other hand to protect proprietary information from public disclosure. That dichotomy is sure to generate a good deal of Congressional debate. The Administration's draft bill and the Rogers bill deal with public disclosure in slightly different ways, though both leave to the Secretary of HEW the ultimate decision on what

NIH grants: OK, but . . .

AFTER a two-year study of the system used by the National Institutes of Health (NIH) to parcel out more than \$1,400 million a year in research grants, an internal NIH committee has concluded that the system is working well, but would benefit from a few changes.

NIH now handles about 15,000 grant applications a year, and funds less than a third of them. They are reviewed first by a study group consisting of a number of scientists working in related fields, which rates the proposal according to its scientific merit and which takes into account such considerations as the track record of the applicant. The proposal, together with the study group's rating, is then passed along to the advisory council of the Institute to which the application has been referred. The council goes into such matters as whether the proposal fits in with the priorities of the Institute, and it recommends whether or not the grant proposal should be funded. The final decision is made by NIH officials. Clearly, the most influential stage of the review is the initial evaluation of the study group.

At present, study groups meet behind closed doors and their recommendations are exempt from general public disclosure. The committee has recommended that the groups continue to deliberate in private, but it has suggested that the groups' conclusions and recommendations routinely be communicated to grant

applicants as soon as the Institute's Advisory Committee has completed its review.

On the question of confidentiality, the committee has also expressed concern about the fact that grant proposals, once they are funded, must be made available to the public, on request, under the Freedom of Information Act. The committee urges passage of legislation protecting grant proposals from public disclosure.

As for the support of unorthodox research, some concern has been expressed that the peer review system favours traditional, conservative research at the expense of creative, unorthodox research. The committee therefore suggests that applicants identify innovative aspects of their research proposals. It also suggests that the study groups prepare a statement pointing out the innovative aspects of a grant application.

Finally, the committee recommends that a formal appeals system be established in NIH through which applicants can appeal the assignment of their proposals. It suggests that an ombudsman be appointed to adjudicate disputes over the handling of grant proposals, and that a Permanent Grants Peer Review Appeals Board be established under the chairmanship of the ombudsman.

The committee's recommendations are now being considered by the NIH Director, Donald Fredrickson. He is expected to endorse most of them.

Colin Norman

information should be made public. The Rogers bill essentially requires the Secretary of HEW to publish in the *Federal Register* details of research projects for which he has issued licences, though he would simply exempt from disclosure proprietary information. The bill under consideration by the Administration, however, would require the Secretary of HEW to make information public only on request and after the researcher involved is given a chance to object if he believes that disclosure would jeopardise commercial interests.

● **The nature of sanctions if violations are discovered.** It is likely that the legislation which will eventually emerge from Congress will empower HEW to inspect facilities, and require reports, health monitoring and so on. The legislation is likely to give the Secretary of HEW the power to revoke licences if violations of the regulations

are discovered, and he would also be able to halt projects deemed to present an imminent hazard. As for the question of liability, the Administration's draft bill would leave that up to state and local laws, while the Rogers bill would allow imposition of a \$1,000-per-day fine for each violation.

Those areas are likely to be the chief focus of discussion in the next few weeks as Congress goes through the unprecedented process of setting federal regulations on an area of basic science. One thing is already clear from the public debate so far, however. As NIH Director Donald Fredrickson put it during last week's hearing "biomedical research is entering a new era in its relationship to society. It is passing from an extended period of relative privacy and autonomy to an engagement with new ethical, legal, and social imperatives under concerned public scrutiny". □

EUROPE

First meeting

THE problem of disclosure of information emerged last week as the most immediate obstacle to progress in formulating European guidelines for recombinant DNA research when representatives of bodies in some 15 European countries concerned with its regulation met in Strasbourg under the European Science Foundation's wing.

The meeting, the first of the newly established European Committee on Recombinant DNA, was from all accounts very useful and friendly but far too short to allow adequate discussion of a heavy agenda. The disclosure issue concerned the details which need to be revealed about particular projects in order that guidelines might be formulated and containment procedures applied without the applicant surrendering scientific or commercial advantage.

The simple origin of the problem is the recommendations of the committee's predecessor. This concluded last year that the recommendations and code of practice of the British Williams report should be adopted as guidelines for recombinant DNA research in Europe. The difficulties arise because those recommendations are general rather than specific, emphasise physical more than biological containment, and operate in a country where agreed standards of confidence obtain and certain patent laws operate.

Since only a body of case law can fill in the grey areas created by the lack of specificity, the argument goes, access to full details of particular cases becomes necessary for everyone using the guidelines, particularly where biological containment allows a drop in the level of physical containment. The trouble is, in Britain disclosure of such detail apparently means forfeiting the right to apply for a patent, and the hard-to-win cooperation of such companies as ICI with Britain's Genetic Manipulation Advisory Group (GMAG) must be on the understanding that revelations to it will not constitute disclosure.

Whether that means that without full disclosure the Williams guidelines could not operate fairly is unclear, but it is apparent that Britain's patent laws, interpreted in this way, could create a problem nationally and certainly internationally. The position is different in the United States, where application for a patent can be filed up to 12 months following disclosure.

In Strasbourg the French and Swedish representatives urged full disclosure, and the representative of Britain's GMAG was not in a position to agree. That means that answers are needed on the precise meaning of Britain's patent laws as they relate to recombinant DNA research before the next meeting expected in September.

No one seems to doubt that there should be a full exchange of information for any European guidelines to work properly. But the potential economic rewards of the work (the bad experience of penicillin is not lost in Britain) are also recognised, and if industry is to be incorporated in the whole scheme without resort to legislation, it may be at the price of not having full disclosure. In that case, the idea of using paradigmatic cases will gather support.

Chris Sherwell

BRITAIN

Sea-bed disposal: safe after all?

An optimistic assessment of the radiological consequences of disposing of solid high-level radioactive waste on the ocean floor is published this week. Chris Sherwell reports

IMAGINE it is the year 2010. The world's nuclear power programme, consisting entirely of light water reactors, had an installed electrical generating capacity ten years earlier of 2,500 GW. The high-level waste produced by that programme has since been cooling in ponds at a reprocessing site, and has now been glassified and put into stainless steel cylinders with 'waste oxide incorporation'. The cylinders—all 72,000 of them—are about to be dumped on the floor of the North Atlantic, in one operation. What will happen over the next 1,000 million years?

An attempt to answer that question is contained in a report out this week from the National Radiological Protection Board (NRPB)* and prepared for British Nuclear Fuels Ltd (BNFL). The subject is especially controversial at the moment in Britain where, apart from the decisions being sought on the choice of the next generation reactor and on the commitment to the fast breeder, the matters of oxide fuel reprocessing and of high-level waste disposal remain unresolved issues.

Just last week, for example, the UK Atomic Energy Authority (UKAEA) attached great importance to the words of Dr Frank Feates, head of Harwell's Environmental Safety Group, at a public meeting in Scotland. The search now being conducted was not for dumping grounds in any particular area, he said; it was to see if unfissured granite, no matter where it was, was a safe structure for high-level waste disposal.

The idea of underground burial, which is being investigated in other countries too and focuses on salt and clay as well as granite, is only one of three options. The others are burial under the ocean floor and placement on the ocean floor, and it is the consequences of the latter alone which the NRPB study examines, even though the London Convention of 1972, which came into force in 1975, expressly forbids such disposal—a point the study mentions in an appendix.

The report endeavours to model the ways in which material deposited on

the ocean floor will re-enter man's immediate environment, especially via his food. The principal route of return it uses is via dispersion in the deep ocean, physical transport to the productive surface layers, incorporation in marine food chains and the consumption of contaminated seafood, but it also considers radiation doses arising from contamination of beach sediments.

Although the report emphasises that only broad conclusions should be drawn, it states that "no overriding reason connected with radiological protection considerations has been identified which would preclude the disposal of suitably conditioned high-level waste on the ocean floor". Other points:

- All the vitrified waste may be expected to be dissolved in about 3,500 years. The heat generated by the wastes would not give rise to "any significant vertical transport mechanism". The amount of activity removed from the water by adsorption onto sediments will not be the major fraction of the total amount released, and will not significantly deplete the quantity of activity in the water, for 10^3 – 10^6 years.

- Assessments, for each of the many nuclides involved, of their changing concentrations over time in surface and deeper waters, and of how these are taken up by plankton, molluscs, crustacea, fish and eventually man, show the relative importance of different routes. The ratio of intake to the maximum permissible annual intake (I/MPAI) attains its highest value for consumption of deep-ocean fish or plankton (neither of which routes currently exist) at times of either 50–100 years or 500–2,000 years. The ratio is 10^{-2} ; for surface fish, the order is 10^{-4} ; other ratios are below 10^{-5} .

- The largest annual collective dose is due to consumption of surface fish (the only intake route actually established), at about 4×10^4 man rems at 50 years from ^{137}Cs and ^{90}Sr taken together. Collective doses at longer times will be of the order of 10^2 – 10^3 man rems per year. The figures would be larger if plankton was established as a major direct food source.

- Predictions of nuclide levels in the sea water do not exceed the level of the nuclides known to be present in sea-water naturally or through fall-out.

The study emphasises that different conclusions might be reached if the waste was separable into short- and long-lived fractions, if a different composition (or even monolithic) glass was available, if better containment materials than steel were used, or if additional barriers, including even the sediment itself, were employed.

*P. D. Grimwood and G. A. M. Webb, *Assessment of the Radiological Protection Aspects of Disposal of High Level Waste on the Ocean Floor*. (NRPB-R48, HMSO, 50p).

BRITAIN

● Conservation is one of the strongest refrains in the UK Department of Energy's (DEN) efforts to produce an orchestrated energy policy. Straight-forward measures like fuel pricing policy and speed restrictions have been buttressed by various schemes like the Save It campaign, the Industrial Energy Thrift Scheme, the Energy Audit Scheme and the Energy Survey Scheme; a recent report on Energy for Industry from Cambridge Information and Research Services has emphasised the importance of conservation in any future strategy; a DEN report on energy elasticities published last week speaks of the savings to the balance of payments through cutbacks in consumption; ministers—last week it was Peter Shore, the Environment Secretary—regularly stress the importance of conservation. And on the international front Britain is now taking part in two of four R&D programmes on conservation through the Paris-based International Energy Agency.

To some it is obvious, to others exotic, but one of the more ambitious schemes mentioned in Britain to encourage conservation has now received an official judgment. It comes from a working party of a special group set up under the aegis of the DEN's Advisory Council on Research and Development (ACORD). The group is the Combined Heat and Power Group and the working party's focus was its domestic application—that is, district heating. The group's main report, which is unlikely to be out this year, will cover industrial applications.

No one has doubted that changing the operation of a conventional power station to produce vast quantities of hot water but less electricity can allow substantial energy conservation. The problem is whether it can be done economically, and last week's report, *District heating combined with electricity generation in the United Kingdom* (HMSO, £3.75), gives a carefully quantified response.

The difficulties in making calculations are enormous. One reason is that it would take some 15 years to develop a heat load suitable for connection to a combined heat and power (CHP) plant. Moreover, a new and expensive system of lines would be necessary to distribute heat to consumers; and even then a great deal depends both on the demand from them (what will their standard of living be?) and their distance from the power station (what planning policies will apply?). The future cost and availability of alternative fuels

and the development of alternative technologies such as heat pumps is also important. So, although the savings in energy are clear and the engineering involved is established, the assumptions that are made when using standard discounted cash flow techniques are critical.



For all this, the working party concludes that with present fuel prices and fuel availability, and with the test discount rate used by the Treasury of 10%, there is no economic incentive to introduce CHP and district heating save for limited high density areas. CHP could be more economic with a reduced discount rate, however, or if fuel prices rose, or both. Fuel saving would amount to 20 mtce compared with the 1972 fuel consumption pattern if CHP plant supplied 25% of the population.

At the turn of the century, on the other hand, when oil and gas will be scarce and expensive, CHP and district heating would be economically attractive compared with direct electric heating or with substitute natural gas produced from coal. About 25 GW less capacity of nuclear power stations would be required compared with direct electric heating if CHP plant supplied 25% of the population, and fuel savings would be somewhere between 7 and 30 mtce.

But if a significant amount of district heating network is to be installed by the first decade of the next century, the working party says, it is necessary to start now, and that means selecting a pilot city for conversion; strong government initiatives are essential if CHP is to develop significantly. Last week, however, Dr John Cunningham, the minister with responsibility for conservation, and Dr Walter Marshall, chief scientist at the DEN, were both at pains to emphasise the political sensitivity of embarking on such a course without proper con-

sideration of the people affected.

● The trouble with the report out this week from the Wellcome Trust covering the 1974–76 period is that there is no indication of the price levels to which its figures refer. Thus the Trust says it allocated over £8.5 million to research in human and animal medicine, which is some 91% higher than the nearly £4.5 million it allocated in the previous 1972–74 period. Inflation was such that £1.35 million of the increase went to meet salary increases for people the trust supports. And it expects an income of £5½ million in 1976–77. But are these and other figures at 1974 or 1976 prices? Answer: the figures are at prices at the time referred to.

The present strength of Wellcome, which is the largest endowed charitable trust supporting medical research in the United Kingdom, is, according to the report, "the result of the expansion of the international pharmaceutical company, the Wellcome Foundation Limited." For the 1976–77 allocation it has had to make a choice between deliberate support of special objectives and *ad hoc* selection from the applications the trustees received. It has opted for a more selective policy for 1977 focusing on such relatively neglected areas as ophthalmic medicine, the vascular system of the human brain in relation to disease, the metabolic effects of infection and the pathology of trauma. There is no increase in funds for projects submitted for *ad hoc* consideration.

● Participants in this weekend's conference in Aberdeen on the changing sea-bird populations of the North Atlantic will gather just five weeks after the latest serious incident involving oil pollution. To the concern of many, at least 700 oiled birds, and possibly twice that number, came ashore near the sites of Yorkshire's cliff-nesting seabird colonies. Faced with the argument that official maps showed no vulnerable bird concentration in the area for that time of year, the Royal Society for the Protection of Birds had to organise its own aerial survey to find and size the oil slick responsible; and the RSPCA actually destroyed some live oiled birds instead of sending them to a new and costly rehabilitation centre. The adequacy of existing maps and the criteria for bird rehabilitation remain important issues to ornithologists. With the subject of pollution on the conference agenda, there could be a bit of a flap in Aberdeen.

Chris Sherwell

USSR

Quality control

Vera Rich writes on the reorganisation of higher degrees in the Soviet Union

THE Soviet media are at present conducting a major campaign eulogising Soviet achievements, as part of the build-up to the forthcoming 60th anniversary of the October Revolution. The achievements of Soviet science feature conspicuously in this campaign. In one field, however, there is little cause for rejoicing, and the press notices strike a critical note—the training of the next generation of scientists and the awarding of higher degrees.

In the Soviet Union, the degrees of Candidate (roughly equivalent to a PhD) and Doctor of Science are awarded not by the universities, but by a special body, the Higher Qualifications Commission (*Vyshshaya Attestatsionnaya Kommissiya*, VAK) of the Ministry of Higher Education. At the beginning of 1976, new regulations for higher degrees came into force, setting new, higher standards and reorganising the structure of VAK. In its new form, VAK consists of a 'Plenum', a 'Presidium' and a 'College', with 34 'councils of experts' dealing with the principal lines of research, and 500 'specialist councils' for assessing degree theses, 150 of these councils being entitled to deal with doctorate theses.

Under the new regulations, a higher standard is demanded, both in the theses themselves and also in the official opponents against whom the theses must be defended. Postulants for a higher degree have, in addition, to fulfil certain non-academic requirements; according to Professor V. G. Kirillov-Ugryumov, the Chairman of VAK, they are expected to "combine a profound professional knowledge with a mastery of Marxist-Leninist theory and with the convictions of an active builder of communist society."

In spite of this emphasis on *partiinost'* (Party-mindedness), the changes were primarily intended to improve the standards of scientific manpower. The first year's work of the reorganised VAK has, however, failed to effect this improvement, and the overall picture suggests incompetence and inadequacy.

According to *Pravda*, a number of the degree councils have not been observing the proper procedure for the defence of theses and have been deliberately lowering their standards for reasons ranging from "bonds of friendship" to the "exploitation of official status" by the applicant. Twenty-one

degree councils have been suspended as a result of such infringements. Nevertheless, during the year, almost 20% of doctoral theses were rejected by VAK.

Theses appear to be of a low standard because more than half the postulants come from industry, having carried out their research while working in jobs which may not necessarily be closely related to their academic work. This is a somewhat delicate matter to broach in the media; Soviet policy constantly urges a closer contact between research and industry, and even so eminent a body as the Siberian Branch of the Academy of Sciences of the USSR was recently censured for slackness in establishing and maintaining such contacts.

However, Moscow radio recently hinted at some of the difficulties facing the young graduate who goes straight into production and attempts, while working, to obtain a higher degree.

Those who embark on such a course, suggested the commentator, should be "surrounded by care and attention". In plants where this had been done, working scientists had obtained degrees not only at the Candidate level but even at the Doctor level. Directors who failed to cherish in this way their ambitious young scientists "display short-sightedness and lack of foresight". The difficulties of combining research with a job, even a job in one's chosen field, were considerable, and accordingly, while in no way overtly criticising official policy, the commentator urged that special attention should be paid to ensuring that more young graduates gain their research experience in the most authoritative establishments.

The current Five-Year plan is aimed at increasing efficiency and quality in all sectors of production, including the higher education 'industry'. The reorganisation of VAK was but one aspect of this drive towards quality. The first results of the new system show that it is not easy to achieve higher standards simply by legislation. □

More give, more take

DR Mikhail Shtern was unexpectedly released last week from the labour camp where he was serving an eight-year sentence for allegedly accepting bribes. The official TASS statement on his release maintained that the sentence was "correct", but claimed that acting on considerations of socialist humanity, and in view of Dr Shtern's poor state of health, the Supreme Court of the Ukrainian SSR had decided to reduce the sentence to two years and nine-months—the term already served.

After his release, Dr Shtern told foreign reporters that he was "absolutely not guilty of anything", and that the accusations against him were made in reprisal for his sons' application to go to Israel. Dr Shtern's release followed a worldwide protest campaign which involved fifty Nobel Prize winners. A special tribunal to examine the Shtern case was scheduled to open in Amsterdam this week.

Dr Shtern's release, however, does not appear to augur a relaxation of pressure on the refusniks. In Moscow, Yosif Begun, an electrical engineer, is in prison awaiting charges of "parasitism", that is, being without visible means of support—a charge which has not been brought against any refusnik for some time. On 15 March, the day after Shtern's release, Anatolii Shcharanskii, the mathematician who has acted as press agent

for the Azbel Seminar and is closely associated with the Moscow 'Helsinki monitoring group', was arrested while actually in the company of two Western journalists who were interviewing him about the Shtern case. Shcharanskii's family has been told that he may be accused of "crimes against the State", an accusation which could lead to a show trial.

Jewish activists in Moscow see the arrest of Shcharanskii as part of a growing campaign of repression against the refusniks. Although there have been recent attacks in the media on other dissidents too, the campaign against the Jews who simply wish to leave the Soviet Union is far more aggressive than that against the Russians, Ukrainians, Lithuanians and others who also urge change.

The anti-refusnik campaign has been particularly directed against the scientists and academics. Searches of unprecedented severity have taken place at the homes of several of them, resulting in the destruction of their books and the confiscation of all papers. At the time of his arrest Shcharanskii, together with Professor Aleksandr Lerner, a cyberneticist, and Vladimir Slepak, an engineer, were attempting to bring a libel suit against the government newspaper *Izvestiya*, which had accused them of spying for the CIA.

Vera Rich

COMECON

● The first meeting of the Heads of the Academies of Science of the Comecon countries took place last month in Moscow. All nine Comecon members were represented, and also Vietnam. Yugoslavia, however, which has a special relationship with Comecon in a number of fields, including science and technology, was not represented. The meeting consisted of an exchange of views on the development of cooperation in solving key problems of "the building of socialism and communism", and increasing the efficiency of scientific research and the implementation of research results in production—this last being the most pressing problem for R&D in all Comecon countries.

Examples of cooperation to date were discussed, including joint programmes for space research, earth sciences, physics, chemistry, and biology. Special attention was given to the role of the scientists in détente and the duty of scientists "recognising their responsibility before history" to use their influence in support of non-proliferation and disarmament. The scientists of Comecon, it was stated, are ready to unite their efforts with those of the scientists of all other countries to work on "major problems of concern to all mankind", such as energy, raw materials, environment, the rational use of ocean resources, and epidemiology. Suiting his tone to the occasion, Mr Brezhnev, in welcoming the delegates to a Kremlin reception, proposed a law of composition for scientific cooperation between socialist countries—individual efforts, he asserted, were not added but multiplied together.

● The recent Romanian earthquake has raised certain doubts concerning earthquake prediction. This is an important topic of research in the Soviet Union, and the countries of Eastern Europe have watched developments in the field closely. In 1976, a Soviet team produced a seismic map of the Balkans predicting probable epicentres for the next 100 years. It would seem, though, that the foretelling of natural disasters is a mixed blessing.

The suggestion of American seismologists that an earthquake of the same magnitude with its epicentre in Romania might be repeated within a few weeks was denounced on Bulgarian radio as "an act . . . beyond all bounds of the admissible and even of the inadmissible in inter-State and inter-human relations", being designed to "create a panic among ordinary people". Whether the Romanians coping stoically with the

aftermath of the disaster were alarmed by the American predictions, or even had the opportunity to hear them, is a moot point; but in Yugoslavia considerable alarm was created in the Krusevac region by radio hams who predicted that an earthquake of even greater intensity was imminent.

The only predictions that have not been pilloried by the East European



media have been the non-alarmist ones—that of the Institute of Geophysics of the Bulgarian Academy of Sciences, which announced comfortably that the periodicity of an earthquake of this magnitude was of the order of 50 years, or that of Professor Bruce A. Bolt of the University of California who, after visiting the site of the Romanian earthquake, pronounced that present scientific methods do not allow one to say whether such a phenomenon might occur in the near future.

According to Dr Negmatullyaev, director of the Soviet Institute of Seismic-Resistant Construction and Seismology, who is at present working on earthquake prediction in Tadzhikistan, current Soviet methods, using boreholes and gas-analysis, allow earthquakes to be forecast with the greatest degree of accuracy. Reactions to the Romanian disaster would suggest, however, that until prediction can be backed up with prevention, the publication of predictions may present a problem in public relations and media management.

Among the victims of the Romanian earthquake were Professor Florin Ciorascu, Corresponding-Member of the Romanian Academy of Sciences; Academician Paul Petrescu, a physicist; Professor Carli Marcu, a pharmacologist; and Professor Marin Lupu, Rector of the Bucharest Academy of Economic Sciences.

Major scientific installations damaged included the National Physics Centre in Bucharest, the Faculties of Chemistry and Medicine at the university and an 'atomic centre'.

● Since the May 1974 visit to Britain of Academician Vladimir A. Kirillin, Chairman of the State Committee for Science and Technology of the USSR, when he declared that industrial fast-breeder generators would not be a practical possibility before the mid-1980s, Western commentators have from time to time suggested that long-range Comecon planning in nuclear power generation is committed to fast-breeders. Such speculations were recently revived at a press conference which Kirillin gave to the heads of foreign missions in Moscow. On this occasion, after referring to the successful operation of the pilot fast-breeder reactor at Shevchenko, Kirillin stated that such reactors would be in industrial use within the next ten years.

Such estimates, however, seem over-optimistic. The recent meeting of the Comecon Permanent Commission for the Peaceful Uses of Atomic Energy in Riga, which reviewed progress to date and approved future plans, paid special attention to the designing of 1,000 MW water-moderated water-cooled reactors. Since this is above the capacity of any reactor scheduled for production in the plans to 1980, any major changeover to fast breeders cannot be expected in the present planning period.

One of the major topics of discussion at the meeting of the Commission was specialisation by the member countries in the production of isotopes, instruments, and equipment for power generation. Yugoslavia, although not a member of Comecon, has a working arrangement by which she participates in a number of Comecon projects, including the Interatomenergi programme for the construction of nuclear power station equipment. Several engineering plants in Yugoslavia are already participating in this construction, including the Litostroj combine in Ljubljana. Litostroj has designed a special circular crane, to operate in complete isolation inside a reactor, to charge it with nuclear fuel and remove the waste products. The equipment is intended for power stations in the USSR; a recent report on Yugoslavia's energy resources and demands makes no mention of nuclear power, but stresses the importance of home-produced coal as the basis of the electrical generating industry.

Vera Rich

IN BRIEF

German nuclear problems

The domestic and international aspects of Germany's activity in the nuclear field have both come in for further dispute recently. Following the visit of the US deputy Secretary of State to Germany a fortnight ago, and the visit to Washington last week of Germany's Foreign Minister, the German position on its controversial nuclear deal with Brazil apparently remains one of determination to fulfil its obligations, in spite of US objections.

Domestic developments have proved less restrained. Violent demonstrations occurred at the weekend outside a nuclear plant in Lower Saxony; and news of a third court case on the construction of a nuclear plant, this time in northern Bavaria, followed the decision last week of an administrative court in Baden-Württemberg to withdraw the construction permit for a plant on grounds relating to safety. A Schleswig-Holstein court blocked

construction of a plant at Brokdorf last month.

Press statement

The long-awaited official announcement finally came late last week: Frank Press, Professor of Earth Sciences at MIT, has been named Director of the White House Office of Science and Technology Policy (OSTP) and science adviser to President Carter. The appointment must be confirmed by the Senate, but no problems are anticipated there. Press's appointment has been a certainty for the past month, but the official announcement was delayed by a lengthy series of security and other background checks which President Carter has insisted be made on top officials in his Administration. Press has been working at the White House, however, attending meetings of Carter's advisers and helping select candidates for other top research jobs in the government.

NSF budget change

In an unusual move, the House Committee on Science and Technology has restored some of the cuts in the budget request for the National Science Foundation (NSF) made by the subcommittee on Science, Research and Technology. The subcommittee had voted to hold NSF's budget increase for fiscal year 1978 to about 7% compared with the 11% boost requested by the Administration. The full committee has restored about half the cuts made by the subcommittee in NSF's basic research and Research Applied to National Needs (RANN) programmes, additions which would give NSF a 9% increase.

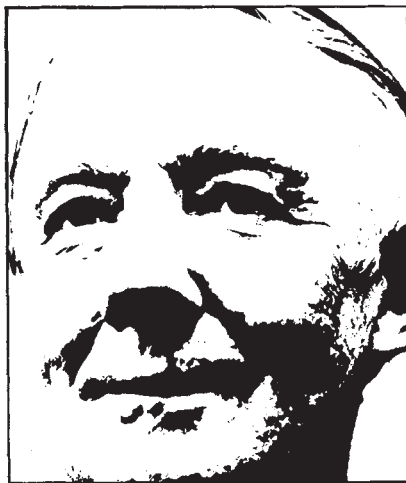
According to staff aides on the committee, the full committee took the unusual step of overturning the subcommittee's recommendations because of recent projections that the inflation rate will be higher than the subcommittee had assumed.

THE most impressive success story in conservation is the recovery of the population of the Antarctic fur seal, described by Nigel Bonner of the British Antarctic Survey in a recent *NERC News Journal*. Before 1791, when the first sealing expedition went to South Georgia, these animals existed in millions. By 1830 they were so scarce that the fur sealing industry stopped. However, they survived in small numbers, perhaps less than a hundred animals, and were officially protected by the British administration of South Georgia in 1907. Most zoologists thought this action was too late and that the species was doomed. But now estimates for 1976-77 suggest that there are over 300,000 and that the population has increased annually by at least 17% for the past 20 years. The seals should soon reach their former level of abundance.

One reason suggested for this phenomenally rapid increase is the ample food supply. Fur seals feed mainly on krill, the shrimp *Euphausia*. This is also the food of the whales which have been so severely reduced in numbers. Successful whale conservation could leave less food for seals. However it seems more likely that man will soon compete for krill, and this could affect both whales and seals. It is therefore interesting to assess the value of this new human food. Would the gain in human nutrition be sufficient to make it worth putting these marine mammals at risk?

The world catch of marine fish for 1974 was estimated at 60 million

tonnes. Biologists believe that it would be possible to harvest almost as great a biomass of krill—at least 50 million tonnes per annum. This would seem at first to add greatly to the food

Fishing line**KENNETH MELLANBY**

resources available for the growing world population. The ocean's contribution to man's food is surprisingly small. Much of the fish is used to feed livestock and pets. When fed to farm animals, the wastage is considerable, and broiler fowls and pigs yield much less protein and energy than the fish supplied to their diet. Even when eaten by man, much of the carcass is wasted. Probably less than 30 million tonnes of actual fish flesh is eaten by

humans each year. Even with the krill, the actual consumption would be unlikely to exceed 50 million tonnes. In terms of energy (calories or megajoules) the total world fish catch would satisfy some 30 million people—less than the annual increase in global population. Even the krill would be consumed by a few month's increase.

Of course, this calculation is, or should be, absurd. Fish is a rich source of high class protein, and the annual catch could supply the protein needs of a tenth of the world's population, and the krill could meet the demands of a further two hundred million individuals. Unfortunately, it is unlikely that it would do so. It is rare for fish to be eaten except by those whose diet already contains sufficient protein. Those who live on staples low in protein, including cassava, plantains and yams, seldom obtain much fish, and it is unlikely that they would benefit from krill. Fish would almost certainly go to increase the luxury eating of man and beast in the richer countries.

We should therefore be cautious before encouraging the exploitation of krill. We see that many of our traditional fisheries are overexploited, and may be destroyed unless internationally conserved. We are very ignorant about the interdependence of krill, seals, whales and man. As the oceans, in spite of overexploitation, are making such a trivial contribution to feeding the world's increasing population, we must realise that most of the food needed must be grown on dry land.

correspondence

Science with public appeal

SIR,—I think your criticism of the BBC's programme, *The Key to the Universe* (3 February, page 393) was unfair to say the least. Nigel Calder did not make any "fundamental mistakes", especially in underestimating the serious mindedness of the audience. Indeed his refreshing attitude encourages the public to take a greater interest in science—we would soon fall asleep (at least I would) if the programme was presented in the way you implied in the article. These two-hour programmes are occasional: they are not the same as *Horizon* and they add a new look to the debate.

I think you are making the fundamental error by assuming that "the uninitiated 99.9% of the audience can only have been thoroughly confused . . .". I did not find the order in which the programme was presented at all confusing. Your leader writer should watch it again as a member of the public and not as a cynical critic.

G. MARKS

Harrogate, UK

SIR—In your editorial, "That was the weak force, that was" (3 February 1977), you make a strong criticism of Nigel Calder's two-hour BBC2 brain-teaser, "The Key to the Universe." You ask, in effect, not only whether it was worth the resources devoted to it, but also whether it might not contribute to the further alienation of many people from science.

These are important questions to which we have no answer. In this regard the BBC fails us entirely. We are not told how much in financial terms such a two-hour programme costs; we do not know whether the key criterion of 'worth' may not be measured solely by audience ratings; and we are completely in the dark about the effect of such programmes on the general viewing audience. That Calder's book of the same title is high in the *Sunday Times* best-seller list tells us nothing about the TV programme except that it surely helps to sell the book.

The non-scientists with whom I watched the programme confessed that apart from the presentation of the 'black holes,' they were baffled. I found the jargon familiar, but I disagreed with some of Calder's emphases. I think he made too much of the tentative, of the as-yet unaccepted, as

in Salam's exposition for example. In that sense, it was difficult to distinguish the 'real' from the 'unreal.'

I have said before that the populariser of science, as he functions today, cannot disseminate the subtle ideas of science; that these really cannot be understood without hard, disciplined effort. We need a research programme on this thesis. The BBC (and ITV) might consider funding that part of it concerned with the presentation of science on TV and radio. Such a proposal concerns the key question of what the presenters of science are really providing, not only in terms of good, entertaining viewing, but also in their contribution to general understanding *en route* to involving the public in decision-making about the use and impact of science in everyday life.

MAURICE GOLDSMITH

Savile Club, London

Tidal energy

SIR—Your leading article (10 February 1977) refers to the 'severe defect' of the periodicity of the tides being not always in phase with the daily load cycle, but nearly 40% of our electrical energy is used for space and water heating where the 'defect' is not at all severe. The use of blocks of tidal energy produced in regular and predictable amounts, both in time and quantity, is now perfectly feasible in the British system, and as installed capacity increases (if indeed it does increase), will become easier rather than harder to absorb. This is without the addition of any further pumped-storage capacity.

The reasons why the French have not proceeded with the *Iles de Chausey* scheme have nothing to do with the foregoing point, since La Rance incorporates a pumped-storage capability to provide power capacity in phase with peak-demand—if that is what the operators require. (Incidentally La Rance is presently using peaking system energy to pump on those occasions when so doing produces more energy overall.) The reasons are both economic and oceanographic, since the inclusion of sophisticated pumped-storage facilities made La Rance so expensive as to discourage further investment in tidal energy, coupled with fears that a much larger scheme might

have reduced the tidal range.

The defects of pumped-storage in estuarial schemes are primarily cost, since such facilities can be provided more cheaply on land (as at Ffestiniog or Dinorwic) or underground, and secondly because the important function of system spinning reserve is unavailable from a tidal pumped-storage plant without loss of available energy. The arguments are all developed in the technical literature and it behoves your leader writer to give it more than a cursory examination before committing himself to a particular solution.

The proposed Severn scheme your article describes is one of at least six proposals made in the last few years, all of which deserve examination in the light of system requirements. It will do the cause of tidal energy no good if a scientific journal of your reputation appears to be prejudging the solution to what is a most complex problem.

E. M. WILSON

Department of Civil Engineering
University of Salford, UK

Geothermal electricity

SIR—Recent studies of potential UK geothermal heat sources (for example, Oxburgh *Nature* **262**, 526; 1976) have been virtually unanimous in stating that temperatures greater than 200 °C would be required from sources intended for electricity generation. A report produced by Patscentre International has shown that, contrary to this general view, it may well be possible to accept a much lower temperature limit. If it can be combined with a fossil-fuel source in an existing or purpose-built power station, geothermal heat in the temperature range of 100–200 °C could be economically used for electricity generation.

In modern steam plants most of the feedwater heat is supplied by low-

Correction

A bad telephone line helped to produce three errors in the article 'Give us a call, says NASA' (10 March, page 112). The Viking chief project scientist is Dr Gerald A. Soffen, not Dr Soffes; the Kyoto meeting will be in the first week of April, not in June, and will be organised by the International Society for the Study of the Origin of Life; and the Tel Aviv meeting in June is the COSPAR and not the pulsar meeting. Sorry.

temperature steam from the turbines. However, this steam, which in a conventional station has been heated by a high-grade fossil fuel, is diverted from work-producing duties in the turbine. If low-grade geothermal heat is used for feedwater heating, the steam could be retained in the turbine, thus increasing both the work output and the cycle efficiency relative to the fossil fuel. The resulting capitalised fuel saving (for the normal rated output) can then be used to pay for the necessary geothermal plant. (I have discussed a similar use for low-grade waste heat in MHD power generation in *Energy Conversion* **8**, 177; 1968.) The amount of geothermal heat that would be usable in this system, which could be applied to either an existing or a purpose-built station, is no more than about 15% of the total heat input; that is, about 200 MW(t) could be accepted by a 500 MW(e) steam plant using geothermal heat at 125 °C. This would give a fossil-fuel saving of 5%.

An alternative purpose-built combined plant, in which the geothermal heat predominates, would use fossil fuel for superheating only. In this case, efficiencies in the region of 100% (relative to the fossil fuel) become possible. For example, 100 MW(e) output could be obtained from 100 MW(t) fossil fuel combined with 360 MW of geothermal heat at 185 °C. This method also has the advantage of low steam wetness, thus appreciably reducing turbine blade erosion, which could occur in a plant that is predominantly geothermal.

The cost benefit analysis proceeds as follows. The present saving on fossil fuel is determined from an annuity-type calculation, taking into account, among other factors, the current fuel cost, inflation of real cost of fuel, interest rate, plant life-time, and increased load factor allowed by increased efficiency.

Thus a capital allowance (per kilowatt of geothermal power) is obtained which can be compared with estimated specific costs of geothermal plant for various conditions.

Results for a purpose-built plant based on a modern conventional power station illustrate the operation of the first system. With the plant situated on hot dry rock supplying feedwater heat at 125 °C, a fossil fuel cost of 9p a therm, and a load factor of 80%, the capitalised fuel saving over 20 years, with a basic interest rate of 10% a year, is £62 per kilowatt of geothermal power. This equals the (1974) cost of the associated geothermal plant (well-head equipment and so on) calculated from fairly conservative data supplied by the Energy Technology Support Unit (Harwell) for a site having a temperature gradient of 45 °C per km. For a similar station situated on an aquifer, the available cost of £62 per kW(gt) would pay for geothermal plant in the upper half of the range of calculated aquifer costs. Of course, the capitalised fuel saving increases with the fossil-fuel cost, the fuel inflation rate (in real terms), and the load factor. Figures for the second type of plant, using 185 °C geothermal heat, show that its unit electricity costs are comparable with those of conventional power stations.

The Patscentre study has shown that geothermal sites that coincide with, or are accessible to, fossil-fuel sources could be of special interest for electricity generation. For instance, the nearness of the Durham hot rocks (granite) to the Durham coal fields should be noted, and coastal sites on the Hampshire sedimentary basin could also be of interest.

R. V. HARROWELL

Patscentre International, UK

Management courses

SIR,—I have long felt it appropriate that the likeness that accompanies

Kenneth Mellanby's articles should be as black and white as the views he expresses! However, the truth about management courses for scientists may not be quite as black as his article (3 March, page 11) might suggest. Five years ago A. J. Lockwood and I started a course in research management (*R&D Management* **5**, 235–238; 1975) for heads of research sections at the Building Research Establishment. It has been held annually since then and now draws participants from a wide range of government research laboratories (both civil and defence). Those who attend appear to find it helpful.

The course was started because many section heads felt the need for some help with the management aspects of their work, a need that was understandably not met by the more general management courses that were then available—and it may be that Professor Mellanby's strictures were directed at such courses. Our courses are deliberately designed for those leading research teams. Perhaps the secret of whatever success the courses have achieved lies in the very fact that we deliberately refrain from any attempt at 'training'. Rather, we try to make available to participants a wide range of conceptual material and practical experience on which they can draw for use in their own circumstances. More important still, we devote about half the course time to fostering, within a carefully thought out series of syndicate exercises, the exchange of the management experience that participants will have accumulated during their working lives. Just as scientists profit considerably from an exchange of their scientific experience, we have found that they can and do receive considerable benefit from an exchange of their (often unrecognised) management experience.

K. ALSOP

Building Research Establishment, Garston, Watford, UK



Competition 12.

A chair in bio-aeronautics has recently been established in the UK. Its occupant is an expert in crop spraying. £10 for the best real or fictitious professorial title. Entries by 20 April to Competition 12, *Nature*.

Competition 11. Readers' contributions offered humorous associations with the work of scientists. Thus: 'Faraday was incapacitated', and so on through a deluge of awful puns. Jim

Watson, according to most competitors, had a guess-what in his neck. Two winners, one brief, one less so.

Brähe thought the paper by Copernicus revolutionary, but pointed out that his argument was essentially circular (M. C. Thorne, Harwell, UK).

On the first day Rutherford reported an immediate disintegration of the Symposium catalysed by Brønsted's acidic comments on Madame Curie's radiant attire. Drs Barnard and DeBailey were further disheartened when Van de Graaff generated a separate reaction from Schiff by referring to her attire as basically colourless. Bragg viewed things from another angle as did Roentgen who saw through it all. Compton recoiled at the prospect of making any statement while Planck and deBroglie also went to some lengths not to make waves—an attitude

that Moseley called uncharacteristic. Wegener asserted that if the rifts continued to grow things would drift further apart. As usual, Heisenberg was uncertain but Coulomb and Nobel were positive that such a charged situation was potentially explosive. Kelvin and Boyle advised that a cooling-off period might reduce the pressures that were building up. Bell thus called on Hubble and Doppler to focus their attention on shifting the emphasis to a series of higher-level discussions as earlier suggested by Balmer and Lyman. Newton seconded the motion and, being short of time, Einstein tried to apply a constant and massive attempt to bring the entire matter to a close. The following day Watson and Crick happily reported that things were taking a turn for the better. (K. M. Towe, Smithsonian Institution, Washington, USA).

news and views

Molecular charmonium?

from Frank Close

A YEAR ago most high energy physicists were agreed that charmed particles must exist, the only problem was that nobody could find any. Then in a study of the debris produced in annihilation of electrons and positrons, charmed mesons called D (1.86 GeV in mass) and D^* (2 GeV) were at last found in the spring of 1976 (see *Nature* 262, 537). Once it was known what had to be looked for and where it could be found old data were studied again and new experiments performed. We now have the surprising result that sometimes too much charm is being found, specifically that when electrons and positrons annihilate at just above 4 GeV an anomalously large production of pairs of charmed D^* mesons is seen. What makes this so remarkable is that the energy of the colliding beams is only just sufficient to produce a pair of 2 GeV mass particles. It is as if the moment that the energy was sufficient to produce them, they just poured out. Why should this be so?

A group of workers at Harvard (de Rujula *et al.* *Phys. Rev. Lett.* 37, 398; 1976) had earlier suggested on statistical grounds that the relative production rates of $D^*\bar{D}^*:D\bar{D}:D\bar{D}$ should be 7:4:1 (there are more degrees of freedom for high spin particles and so the spin 1 D^* is more readily produced than the spinless D). The observed rate of $D^*\bar{D}^*$ production is far in excess of this and so it seems that there is something in the underlying dynamics of the production process that is enhancing the D^* pairs.

When this discovery was reported at a seminar at Harvard, two groups of theorists suggested that charmed molecules might exist (de Rujula *et al.* *Phys. Rev. Lett.* 38, 317; 1976; P. Cox *et al.* Harvard preprint, 1976). Their idea was that in the electron-positron annihilation, in addition to resonances of charmed quarks and charmed antiquarks (like the ψ meson at 3.1 GeV mass) there might be found resonances of charmed mesons and their antiparticles. If such a $D^*\bar{D}^*$ resonance occurs at 4.03 GeV, then this could explain the copious production of D^* and $\bar{D}^*$ at this energy.

But critics say that this is tautology. To

say that there is a $D^*\bar{D}^*$ resonance is simply equivalent to saying that the $D^*\bar{D}^*$ are produced more often than expected. Since no completely satisfactory mechanism has yet been given for producing such a resonance the copious production of D^* pairs has still not been explained. Supporters argue, however, that the idea can be tested and so has some merit. If energies away from 4.03 GeV are looked at the D^* should be less copiously produced.

Whether or not the idea is right it has suddenly gained wide attention in the high energy community although it is by no means new. Several papers have appeared during the past two years and been largely ignored. They have implied or explicitly suggested the existence of charmed molecules as the consequence of investigating the broader issue of the binding of quark states, and it is in connection with this broader issue that the above discoveries might turn out to be significant.

Multiquark hadrons

Mesons are formed when a quark (q) and an antiquark ($\bar{q}$) bind together with orbital angular momentum (L). The mass of the resulting meson can be estimated from the rough rule that each quark or antiquark contributes around 300 MeV and each unit of L -excitation gives around 500 MeV. The quark has spin 1/2 and so the quark and antiquark can spin parallel (spin 1) or antiparallel (spin 0). Then, just as in atomic physics, the spectrum has the form $^1S_0, ^3S_1; ^1P_1, ^3P_{0,1,2}$ and so on, and a hyperfine splitting causes the singlet states to be lighter than the triplets (for example, the pion 1S_0 is lighter than the ρ 3S_1).

These rules mean that the lightest vector particles will be around 700 MeV since they are the $^3S_1 q\bar{q}$ states. Of particular interest are the scalar states 3P_0 . From our rule these states will have a mass around 900 MeV since the hyperfine splitting has pulled them down relative to the average mass of about 1,100 MeV for the P-wave $q\bar{q}$ system. Hence roughly half of the mass has arisen from the two quarks intrinsically and half is from the P-wave excitation.

One can also form scalar states (0^+) from $qq\bar{p}\bar{p}$ where now all of the quarks are in S waves. The extra mass of 600 MeV associated with the two additional quarks is compensated for by the absence of the P-wave excitation and so the mass of this four quark scalar meson can be comparable to the two quark state. In fact the hyperfine splitting in the four quark state tends to be larger than in the two quark and an explicit calculation by R. Jaffe of MIT (*Multiquark hadrons*, SLAC Publications, 1772, 1773) found that the $qqq\bar{q} 0^+$ is lower in mass than the $q\bar{q}$. Astonishingly he presented a good case for believing that the familiar 0^+ mesons are really four quark states and not $q\bar{q}$! The implication is that four quark states have to be taken seriously.

For states containing a charmed quark (c) the same rough and ready rules seem to apply except that one has to allow 1.5 GeV for each charmed quark instead of 300 MeV. The lightest mesons $c\bar{q}$ will be expected around 1.8 GeV and the lightest charmonium states $c\bar{c}$ around 3 GeV. Both of these are realised in the data. Furthermore the P-wave $c\bar{c}$ states $^3P_{0,1,2}$ do seem to spread around 3.5 GeV in line with the rules.

Now replay Jaffe's game but with charmonium. The 0^+ ($c\bar{c}q\bar{q}$) state could be as low as 3.5 GeV (comparable to 0^+ ($c\bar{c}$) at 3.4 GeV): it is possible that a mysterious state at 3.46 GeV could be such a beast. The states that will be produced in electron-positron annihilation are vectors (spin 1, negative parity). If made from four quarks then a P-wave excitation is required and hence the mass rule implies another 500 MeV compared with the S-wave $c\bar{c}q\bar{q}$ (3.46 GeV?) or 800 MeV above the S-wave vector $c\bar{c}$ at 3.1 GeV. Either of these estimates suggests that the 4 GeV region is the place to look.

Very soon after the ψ was discovered Iwasaki in Japan suggested that $c\bar{c}q\bar{q}$ states might exist and even predicted a $c\bar{c}c\bar{c}$ at 6 GeV. The possible existence of such a state is controversial. When the plethora of dips and bumps were discovered in the 4 GeV region of the e^+e^- annihilation cross section, Rosenzweig (*Phys. Rev. Lett.* 36, 697; 1976) and also

Bander *et al.* (*Phys. Rev. Lett.* **36**, 695; 1976) independently suggested that four quark states $cc\bar{q}q$ had been manifested. At the time of these works the charmed states $c\bar{q}$ had not been isolated!

After the D and D^* charmed particles had been found with masses around 2 GeV, Nussinov and Sidhu at the Fermi National Accelerator Laboratory near Chicago explicitly investigated the possibility that charmed mesons might bind together in a manner analogous to the way that nucleons bind to form deuterium. Their explicit calculations (Fermilab preprint, August 1976) indeed showed that a loosely bound $D\bar{D}$ state could exist near to 4 GeV and they speculated that a similar occurrence might also happen for DD^* and $D^*\bar{D}^*$. In the light of the recent developments a report on their calculations of the $D^*\bar{D}^*$ possibility is eagerly awaited.

If $c\bar{c}q\bar{q}$ states are indeed around the 4 GeV mass region then several questions come to mind. Once formed how do they collapse? Is the decay to $c\bar{q}$ and $q\bar{c}$ pre-

ferred to cc and qq ? The latter decay pattern has been called "charm burning" and has been investigated in particular by Okun and Voloshin in the Soviet Union. There is no obvious presence of $\psi(c\bar{c})$ in the debris produced at 4 GeV so this latter mechanism may be unimportant. Is this a problem? In the absence of dynamical information on the binding of the four quark states this is perhaps an open question. Are the four quark states $(cc)(q\bar{q})$ necessarily the same as $(c\bar{q})(q\bar{c})$?

If the quark model is relevant to nature then these four quark states seem to be unavoidable. With all the current interest in the 4 GeV region of energy in electron-positron annihilation one might hope that the first evidence for them may show up here. If the ideas of Iwasaki, Rosenzweig, Bander and many others are right, then it is possible that such a state has already been seen, in which case a whole new spectroscopy of molecular charmonium awaits discovery in addition to the "traditional"(!) charmonium now being unravelled. $\square$

mutants as for the wild type. The unit cell of fibres maintained at 92% R.H. is orthorhombic with dimensions $a = 2.030$ nm, $b = 1.178$ nm, c (fibre axis) $= 3.044$ nm. This contains four hexasaccharide units, one along each c edge and one central, and the space group $P2_12_12_1$ dictates that the central chain lies antiparallel to the side chains. The conformation of the chain is presented in Fig. 1. The main chain lies in a two-fold helix and the side chains are folded back to lie parallel to the main chain. Of the 15 potential hydrogen donors in each asymmetric unit, only five have been explicitly identified as participating in hydrogen bonds within and between chains. Presumably the other 10 may represent hydrogen bond bridges utilising a single water molecule between the chains. When the fibres are dried over silica gel, the unit cell is reduced in volume by contraction in a (to 1.73 nm) and b (to 1.02 nm) and then several of these bridges appear to be converted into direct intermolecular hydrogen bonds. *In vivo*, this highly hydrated polysaccharide is attached to the exterior of the cell in all three forms either as a capsule or contributing to long loose strands which project outwards.

Its functions seem to be many. *E. coli* phage 29 is highly specific for this capsule, cleaving β -D-glucosido-(1 $\rightarrow$ 3)-D-glucuronic acid bonds exclusively and is dependent on the presence of the pyruvate ketal. The authors consider that the viral glucanase may move along the polymer strands, cleaving the glycosidic bonds and thereby opening a path for the phage head. Some particular conformational aspect of the polysaccharide, still to be determined, may then direct the phage to a particular site. Otherwise, the capsular polysaccharide prevents bacteriophages specific for cell wall receptors from infecting the cell and protects the bacteria from phagocytosis by macrophages; the structure so far elucidated gives no clue to the molecular mechanisms involved.

Although Morris *et al.* (*op. cit.*) refer to the structure determination of the polysaccharide they discuss, the details have not yet been reported and their paper deals exclusively with the behaviour of the chain molecules in solution, as judged by temperature effects on optical rotation, circular dichroism, proton magnetic resonance and viscosity; to this extent it complements the findings of Moorhouse *et al.* They deal, however, with different polysaccharides, the extracellular polysaccharides of *Xanthomonas* spp., pathogens responsible for blight disease of a number of important crop plants. These have a range of distinctive rheological properties which give them a variety of industrial uses. The primary structure consists of a backbone of repeating $[\rightarrow 4]\text{-}\beta\text{-D-glucopyranose-}(1\rightarrow 4)\text{-}\beta\text{-D-glucopyranose}(1\rightarrow)]$ as in cellulose, with a substituent $[3\rightarrow 1)\text{-}\alpha\text{-D-}$

Versatile polysaccharides

from R. D. Preston

THE recent explosion of information on the detailed structure of a variety of polysaccharides, largely at the hands of four groups of workers, provides a salutary reminder, for those who need it, that these substances are to be reckoned with as well as proteins and nucleic acids. Broadly speaking, polysaccharides serve two main functions in living organisms. They largely determine the mechanical properties of supporting tissues, such as cell walls in plants and connective tissue in animals, and on the other hand, serve as recognition and receptor sites, a function particularly well-studied in bacteria, in which polysaccharides may be involved in recognition and adsorption of bacterium and phage, and of bacterium and host.

It is this function of polysaccharide that has attracted the attention of two groups of workers, D. A. Rees and colleagues in the UK and S. Arnott and coworkers in the USA. Two recent publications present the first detailed conformation of branched polysaccharides and give structural information relevant to their function as agents of recognition (Moorhouse *et al.* *J. molec. Biol.* **109**, 373; 1977; Morris *et al.* *J. molec. Biol.* **110**, 1; 1977).

Structural information on polysaccharides has been scanty until comparatively recently because no polysaccharide gives enough X-ray evidence to allow a complete determination of

structure. The recent spectacular advances stem, not from advances in the technique of X-ray analysis itself, but from a combination of X-ray data with conformation theory as developed by Ramachandran and his colleagues and modified particularly by Arnott and his group (Ramachandran *et al.* *J. molec. Biol.* **7**, 95, 1963; Arnott & Wonacott *J. molec. Biol.* **21**, 371; 1966; Arnott & Scott *J. Chem Soc. Perkin* **324**; 1972; Arnott *et al.* *J. molec. Biol.* **90**, 253; 1974). Both Moorhouse *et al.* and Morris *et al.* use this approach in defining the structure of their polysaccharides and, with the former, in presenting computer-drawn models complete with the necessary hydrogen bonding within and between the molecular chains and the positions of included water and anions.

Moorhouse *et al.* deal with a capsular polysaccharide from *Escherichia coli* strain Bi161/42(09:K29(A):H) in the form of chains of hexasaccharide repeating units $[-2\text{-}\alpha\text{-mannopyranose-}(1\rightarrow 3)\text{-}\beta\text{-D-glucopyranose-}(1\rightarrow 3)\text{-}\beta\text{-D-glucuronic acid-}(1\rightarrow 3)\text{-}\alpha\text{-D-galactopyranose-}(1\rightarrow)]$ with a disaccharide $[\beta\text{-D-glucopyranose-}(1\rightarrow 2)\text{-}\alpha\text{-D-mannopyranose}]$ attached by a $1\rightarrow 4$ link from the mannopyranose residue to the glucuronic acid. Pyruvate is attached to every terminal D-glucose residue. This polysaccharide has been isolated from the serotype K29 and from two mutants, M13 and M41. The structure turns out to be the same for the

mannopyranose-6-O-acetate-(2→1)-β-D-glucuronic acid-(4→1)-β-D-mannopyranose] on every other glucose residue. Each side chain appears to carry O-acetate, but in addition pyruvate is found attached to mannose residues though only to about one third of this extent.

The crystallographic evidence (not yet reported) is said to imply that the side chains, like those of the *E. coli* polysaccharide, fold down to form favourable noncovalent interactions with the main chain, and the molecule as a whole constitutes a five-fold helix in the form of a rigid rod. All the spectroscopic evidence with solutions points to a change from an ordered form to a disordered form as the temperature is raised. The transition temperature is independent of concentration and from this it is argued that the ordered form is a single-chain species (though it is agreed that this is not yet completely certain) and that this ordered form could have the same conformation as in the fibre. This last consideration is one that vexes all attempts to understand solution properties, and properties *in vivo*, in terms of the conformation of the solute molecule when in a crystallite, and it cannot be said that the authors give direct proof of this correspondence. If the correspondence is acceptable, then the rheological behaviour can be understood in terms of weak interactions between rigid rods forming a tenuous three-dimensional network which is dispersed as the temperature rises.

A clue to the function of the polysaccharide of this pathogen is given by the observation that when mixed with even low concentrations of galactomannan the mixture may gel even though neither polysaccharide alone does so. The interaction is thermally reversible, and the gel is stronger when the ratio of mannose to galactose is 4:1 than it is at a ratio of 3:1, and when the ratio is 2:1 the mixture does not gel at any temperature. From this it is deduced that non-covalent binding occurs between the unlike chains of 'cellulose' and mannan. This could be regarded as 'recognition' of one polysaccharide by another. The interaction is not confined to galactomannans, however, but occurs with other polysaccharides with the β-1, 4-linked backbone including cellulose derivatives (Dea, Morris & Rees, *in the press*). There are therefore in the walls of the host plant a number of polysaccharides to which the bacterial polysaccharide might become attached and the suggestion made by the authors that this might constitute a molecular 'holdfast' securing the pathogen to the cell surface is not without its attraction. In going further by suggesting that this mechanism might ensure recognition of particular sites on particular plants the authors are, however, clearly going beyond the evidence. There seems no reason at the moment to believe that this association between polysaccharides

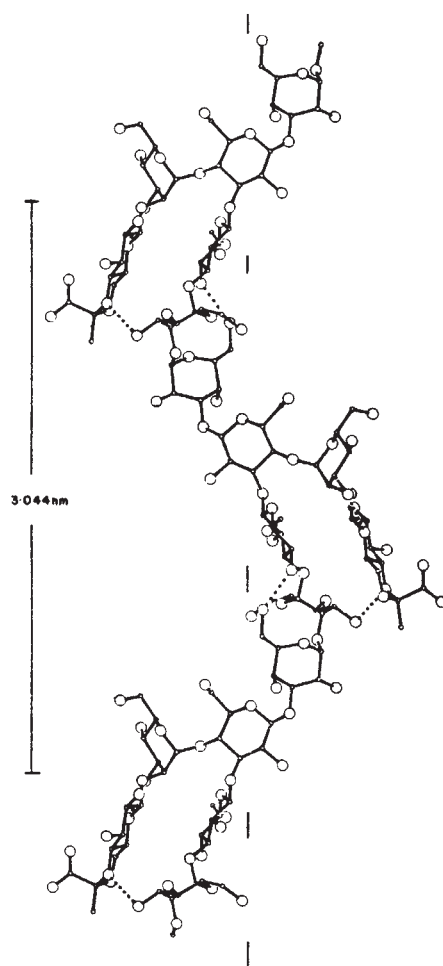


Fig. 1 Molecular conformation of the M41 polysaccharide. Open circles, oxygen; hydrogen omitted. Broken lines denote hydrogen bonds.

would confer sufficient specificity and further evidence is needed on the polysaccharide composition of cell wall surfaces in host and non-host plants before accepting this as 'recognition'.

It is welcome that two groups of workers have now delineated the conformations of two branched polysaccharides and that, in spite of the different chemistry, there is an overall similarity between them. This will one hopes stimulate closer attention to the other partner in the proposed recognition mechanisms, namely to the possibility of variations in the matrix substances of the walls of host and non-host cells, and, for *E. coli*, to the structure of the viral glucanase of phage 29 as a check on its compatibility with the function claimed. More remotely from the field of the present two papers, but of equal urgency, success with these two polysaccharides should stimulate attempts at the conformation of branched polysaccharides in higher plant cell walls, to the benefit of cell wall mechanics both in cell growth considerations and in, for example, timber technology. The importance of these papers ranges well beyond the confines of bacterium/host recognition.

Laser dissociation

from Graham Richards

LASERS offer to chemists the possibility of controlling the specificity of chemical reactions by choosing the energy of the laser beams so as to cause the rupture of particular chemical bonds. Most attractive from this point of view is the prospect of isotopic separation by laser dissociation of polyatomic molecules. Powerful CO₂ infrared lasers have been used successfully to produce enrichment of boron isotopes from BCl₃, sulphur from SF₆ and more recently amongst heavier nuclei, osmium from OsO₄ (Ambartzumian *et al.*, *JETP Lett.* **22**, 43; 1975) and molybdenum from MoF₆ (Hudson *et al.* *Electro-Optical Systems Design Conference*, 1976). The hope is that the technique may lead to a method of separation of uranium isotopes by laser action on UF₆.

The dissociation of a molecule by infrared radiation, whose photons have enough energy to increase vibration but far too little to break a bond, can only succeed if many photons are absorbed so rapidly that energy is not lost on collision between molecules. The high power density of the CO₂ laser enables these conditions to be met. The sharp frequency of the laser may fit with a vibrational frequency of one isotopic species, but not with another since in molecules, as in all vibrating systems, the vibration frequency decreases with increasing mass of the oscillator. Once started up the vibrational ladder by absorbing several photons, one isotopic molecular species may be decomposed leaving an isotopically enriched mixture.

Despite a great deal of work, many questions about the mechanism of this potentially valuable technique remain. How can a molecule absorb a number of quanta of the same energy even though the vibrational energy levels converge as dissociation approaches? It could be due either to multiphoton absorption (several photons absorbed at the same instant) or to the use of rotational energy levels which give fine structure to the vibrational energy ladder. Theoretically the former hypothesis seems more likely (Bloembergen *Opt Commun.* **15**, 416; 1975), but even in the case most studied, SF₆, the mechanism of multiphoton absorption is far from completely understood. What are the dissociation products; how many photons can a single molecule absorb beyond the dissociation limit, and how is the excess energy distributed among the fragments? What is the effect of molecular collisions and is there an activation energy

barrier beyond dissociation? Many of the unanswered questions are clarified by the elegant experiment of Coggiola *et al.* (*Phys. Rev. Lett.* **38**, 17; 1977) on the multiphoton dissociation of SF₆. They establish that the molecule does accumulate energy by sequential absorption of photons and provide many details of the mechanism.

Their experiment involved crossed molecular and laser beams; an SF₆ beam formed by expansion was crossed with a pulsed CO₂ laser beam (1 J per pulse). The fragments produced by multiphoton dissociation of the SF₆ were analysed with a quadrupole mass spectrometer whose position could be varied to measure the angular distribution of fragments.

The observation of dissociation products from the beam interaction region confirms that the multiphoton molecular dissociation is indeed a unimolecular process since there is no possibility of collision.

The SF₅⁺ ion signal is always the most intense and no F₂⁺ was detectable. Ions with one, two or three fluorines removed and F⁺ all have the same parent molecule, SF₅. The major products are thus SF₅ + F, rather than

SF₄ and F₂. The possibility that clusters or van der Waals dimers of SF₆ are the origin of the fragment signals was ruled out by studying the dependence of the SF₅ signal as a function of SF₆ beam saturation pressure when no increase in intensity beyond that due to increased beam flux was seen.

The translational energy released in the dissociation was found from the angular distribution of products using conservation of energy and linear momentum. It proves to be very small (< 6.3 kJ mol⁻¹), implying that there is no substantial barrier beyond the dissociation limit. The activation energy required beyond dissociation is less than 70 cm⁻¹.

The laser affords the possibility of varying intensity, power, polarisation and wavelength, and the SF₆ beam may be excited both vibrationally and rotationally. Further experiments should thus yield detailed information about dissociation dynamics and internal energy distribution and energy transfer of an excited molecule before dissociation. This should in future give a much better understanding of the mechanism of multiphoton dissociation. □

In control experiments where only ³H-UTP is present some terminal addition to 5S RNA occurs and an appropriate correction has to be made. The addition of exogenous polymerase III results in a <50-fold increase in 5S RNA in the transcript is about the same as for the endogenous activity and is asymmetric. In contrast exogenous polymerase I did not stimulate 5S synthesis even though there was a net stimulation of total RNA synthesis above endogenous levels. In all cases a small but indeterminate amount of spacer transcription is seen. It is clear that the transcription of 5S RNA from *Xenopus* chromatin requires the mediation of a specific polymerase. This conclusion is reinforced when chromatin is transcribed with mouse plasmacytoma polymerase II and *E. coli* RNA polymerase. In neither case is there a specific stimulation of 5S RNA synthesis.

In another series of experiments purified *X. laevis* DNA was transcribed in identical conditions with *Xenopus* I, III and *E. coli* polymerase. In each case only about 1% of the transcript was 5S RNA and copying was symmetrical. Taken together these findings suggest that chromatin-associated proteins are required for the selective and asymmetrical transcription of 5S RNA genes in amphibian oocytes and that a specific polymerase is required to effect this. It is clear from the data that a high degree of proper initiation and termination occurs. The situation with other cell-free systems is unfortunately not so precise. A number of laboratories are currently investigating the control of specific genes in a variety of animal systems (such as globin, ovalbumin and histone genes) by analysing the *in vitro* transcripts from isolated chromatin.

In nearly all cases *E. coli* RNA polymerase rather than the homologous polymerase II is used because of the relative inactivity of the latter when added exogenously. The transcripts are usually analysed by hybridisation to complementary DNA made from the appropriate mRNA. It could be argued that the analysis is geared to the biological expectation of the template rather than to the fidelity of the polymerase, however it is clear from these experiments that bacterial polymerases can transcribe chromatin in a tissue-specific fashion. The question of how specific the overall transcription process is in these systems has been studied recently by Biesmann *et al.* (*Biochemistry* **15**, 4356; 1976) by transcribing *Drosophila* chromatin with *E. coli* polymerase and hybridising the RNA to cDNA made to the polyadenylated nuclear RNA of the tissue. In summary these results showed that

Specificity of chromatin transcription

from Stewart Gilmour

MUCH of the information about gene regulation in animals has come from experiments in which chromatin is transcribed *in vitro* with exogenous RNA polymerase. In many cases *Escherichia coli* RNA polymerase is used and it is pertinent to ask whether the transcriptional patterns reflect the regulatory features of chromatin or an incompatible marriage of bacterial polymerase and animal chromatin. Could it be that a bacterial enzyme may not recognise some active sequences in chromatin or perhaps transcribe other sequences which are normally inactive *in vivo*? An insight into this question has been provided recently by Parker and Roeder (*Proc. natn. Acad. Sci. U.S.A.* **74**, 44; 1977) for the particular case of 5S RNA gene transcription in isolated *Xenopus* oocyte chromatin. Normally *in vivo* these reiterated genes are transcribed by RNA polymerase III and in immature oocytes this accounts for a substantial proportion of the total RNA synthesis. The spacer region between the gene copies is apparently not transcribed.

In these experiments chromatin isolated from immature *X. laevis* oocytes was transcribed *in vitro* with exogenous RNA polymerase I and III from *X. laevis* ovaries, polymerase II from

mouse plasmacytoma cells and *E. coli* RNA polymerase. The authors devised a hybridisation assay which analysed the fidelity of the chromatin transcripts with respect to sense strand, anti-sense strand and spacer region copying of the 5S gene. This was achieved by two separate hybridisations. First, transcripts labelled with ³H-UTP were hybridised to plasmid DNA containing homologous *X. laevis* 5S DNA in the presence and absence of saturating amounts of unlabelled 5S RNA. The levels of hybridisation in the two cases indicated the degree of sense and anti-sense strand copying and by simultaneous inclusion of standard ³²P-labelled 5S RNA in the hybridisation reactions an accurate estimate could be made of the total 5S RNA transcript synthesised. In the second analysis transcripts were hybridised to plasmid DNA containing 5S DNA from *X. mulleri*. Since only the 5S gene regions and not the spacer regions are homologous between *laevis* and *mulleri* any aberrant transcription of spacer will not score as hybrid in this case.

Incubation of *X. laevis* chromatin without additional polymerase reveals endogenous polymerase III activity. About 40% of the transcript is 5S RNA transcribed from the sense strand.

while most of the sequences represented in the cDNA probe were also found in the transcript some aberrant transcription also occurred. While it is possible to demonstrate a fair degree of the *in vivo* transcription pattern *in vitro* by this approach it has to be conceded that some genes, like the 5S gene, may be subject to finer levels of control which may not be recognised by heterologous polymerases. Gross controls such as heterochromatisation, acting at the structural level, might determine which tissue-specific genes are transcribed from chromatin *in vitro* and may require little discrimination on the part of the polymerase for the selective transcription of these genes.

Difficulties in solving heparin structure

from Edward A. Johnson

HEPARIN has now been in clinical use for 40 years, but the achievement of anything like a complete understanding of its anticoagulant action still seems a long way off. The difficulties centre on our ignorance of the structure of heparin; or perhaps one should say structures, for it is clear that not only is the most thoroughly purified material a mixture of many compounds, but various components of the mixture exert different effects within the coagulation 'cascade'—that insecure sequence of enzyme reactions which may sometimes begin with the activation of Hageman factor and (with more confidence) may be said to end with the cross-linking of fibrin by activated fibrin stabilising factor to form an insoluble clot.

A matter in dispute since the discovery of heparin is its native form in tissue; more specifically, in the granules of mast cells which are its only certain source, although Jaques and coworkers (*Lancet* **i**, 411; 1977) have recently suggested that it may be present in other kinds of cell. Silbert, Austen and coworkers (*J. biol. Chem.* **252**, 518; 1977) have now consolidated earlier work by Horner (*J. biol. Chem.* **246**, 231; 1971), confirming that, in some tissues at least, native heparin has a high molecular weight of the order of 750,000 to 1,000,000. These large molecules are made up of a number of chains more nearly the size of commercial heparin (5,000–40,000) linked to a common core of uncertain character by polypeptides which, surprisingly, are resistant to all the pro-

Triassic extinction or Jurassic vacuum?

In his article "Triassic extinction or Jurassic vacuum?" (*News and Views* **265**, 402; 1977), Milner refers briefly to my work on a Middle Jurassic microvertebrate locality in Oxfordshire. (*Science* **194**, 1053; 1977). He expresses hope that it and other new localities in Queensland, India and the Isle of Skye may provide data on the times of extinction of the procolophonids, rhynchosaurs, thecodonts, dicynodonts and temnospondyl labyrinthodonts, all of which are conventionally supposed to have died out at the end of the Triassic.

The Oxfordshire site has yielded isolated bones and teeth of mammals, therapsids, theropods, ornithischians, ?sauropods, pterosaurs, crocodilians, lepidosaurs, chelonians, anurans and actinopterygians, but in spite of a deliberate search, no teeth of labyrinthodonts have yet been found. The fragmentary condition of the material would not permit ready identification of the other Late Triassic groups to which Milner refers even if these were present. The diversity of the vertebrate fossils from Oxfordshire indicates that a wide range of vertebrate palaeoenvironments fell within the catchment area of the deposit, and that therefore the absence of the labyrinthodonts in the Late Bathonian is real rather than apparent.

ERIC F. FREEMAN

teases to which they have so far been exposed. However, treatment with aqueous alkali, a procedure often used in commercial heparin extraction, readily releases the chains from the core to give a product which is presumably the equivalent of the heparin of commerce.

Heparin, as a strongly anionic polysaccharide, exerts its effects by binding specifically to basic regions of certain proteins, in particular to antithrombin III (see Seegers, Rosenberg and other workers in *Adv. exp. Med. Biol.* **52**, 1975), the inhibitory action of which on serine esterases in the coagulation cascade is thereby greatly accelerated. It is reasonable to suppose that binding will depend on the presence of appropriate sequences peculiar to the heparin chains, since related acidic glycosaminoglycans and other polysaccharides, whether artificially sulphated or not, are all far less effective than

heparin in the test systems used. The difficulties of identifying these sequences are very great, principally because heparin is always heterogeneous, a complete obstacle to the quasi-statistical methods used in sequencing proteins. Also, hydrolytic degradation is invariably accompanied by far more damage to the released fragments than occurs with peptides, and enzymic methods have to be used.

Useful initial fractionation may be carried out by making use of protein-heparin binding, and, by adsorption on matrix-bound antithrombin or by related procedures, several groups of workers (Lam *et al.* *Biochem. biophys. Res. Commun.* **69**, 570; 1976, Höök *et al.* *FEBS Lett.* **66**, 90; 1976; Andersson *et al.* *Thromb. Res.* **9**, 575; 1976) have concentrated heparin fractions with strong antithrombin binding from various commercial preparations. Such high-affinity material has been called, too loosely, 'active' heparin in contrast to the more weakly bound 'inactive' heparin (which seems to be indistinguishable in its general physicochemical properties), but it has yet to be proved that antithrombin-binding capacity *in vitro* is the only significant criterion for anticoagulant activity in heparin. It may be important; but there is certainly evidence that, at least *in vitro*, the antithrombin-binding capacity of certain heparin fractions does not follow their activity in other and presumably unrelated stages in the coagulation cascade (Andersson *et al.* *Thromb. Res.* **9**, 575; 1976). High-affinity heparin is still very heterogeneous, but Lindahl's group, with Linker (*FEBS Lett.* **69**, 51; 1976), has taken a further step by adsorbing heparin fractions on antithrombin-Sepharose and incubating the complex with heparinase. The segments of the heparin chains bound to the protein were not attacked, but could be displaced in the usual way by increasing the ionic strength. The material isolated in this way had molecular weights of 2,000 to 5,000 (6–16 monosaccharide units), depending on the heparin starting material, but was still heterogeneous.

This kind of approach, ultimately identifying and comparing the heparin segments that are most strongly bound to various proteins involved in coagulation, is difficult and laborious; few of the other relevant proteins are as easy to isolate or as stable as antithrombin III. Nevertheless, it is probably the best hope so far of gaining a proper understanding of heparin anticoagulation. Additional help can come from fluorescence and circular dichroism effects of heparin-protein interactions and also from X-ray diffraction studies on heparin salts—which may give some

insight into heparin conformations in solution—but so far as can be seen the obstacle of heparin heterogeneity must in every case limit the information that can be gained. Any useful procedure should include studies of heparins from different tissues and species; lung and mucosal heparins have been known for many years to differ chemically to a small but consistent extent (do the mast cells differ?) and, since this difference is now known to be paralleled by differences in relative activity at different stages in the coagulation cascade, its significance needs to be examined.

One of the driving forces behind this work has been the importance of heparin in clinical practice, including, especially, its use for antithrombotic prophylaxis following major surgery (see *Heparin: Chemistry and Clinical Usage* (eds Kakar and Thomas) Academic, London, 1976). It is generally agreed that in this context, where prevention of venous thrombosis must not be accompanied by a tendency to haemorrhage, antithrombin potentiation, as determined by the so-called anti-Xa assay, is as yet the best criterion of efficacy. A recent observation of Thomas and coworkers (*Lancet* **i**, 120; 1977) is therefore disturbing; it demonstrates that a semi-synthetic heparin-like compound, which has negligible anti-Xa activity *in vitro*, nevertheless shows excellent antithrombin potentiation in the human subject. In heparin therapy the correlation of assay results *in vitro* with consequences *in vivo* has been taken on trust perhaps more than in other fields, and this will also need reconsideration. □

Probing facilitated transport

from S. J. Smith

INSULIN acts on fat cell plasma membranes to promote glucose uptake and its stimulation of the utilisation of this sugar seems to be amplified by the production of a 'second messenger'. The glucose transport system in adipocytes has been well characterised as a carrier-mediated facilitated diffusion mechanism which shows bidirectionality, stereospecificity and saturation kinetics. However, little is known about the system in structural terms.

Aryl azide derivatives provide a powerful technique—photo-affinity labelling—for identifying sites of biological importance. Irradiation of the probe causes photolysis of the azido moiety producing a highly reactive nitrene, which can react covalently

with any groups that lie within reach. In order to provide any interpretable information, the reagent must be shown to bind at the required locus before photolysis. Singer and coworkers have recognised the possibility of 'pseudo-affinity' labelling, which could occur, where the photolytic intermediate is able to bind reversibly to and dissociate from the active site before a covalent bond is formed. In such cases, the process would be analogous to affinity labelling. Attempts have been made to use this technique to label biological receptors in various systems.

Recently, a photoreactive aryl azide derivative of glucose, N-(4-azido-2-nitrophenyl)-2-amino-2-deoxy-D-glucose has been used to label adipocyte membranes (Trosper & Levy *J. biol. Chem.* **252**, 181; 1977). In the absence of light it has been shown to inhibit the uptake of D-glucose and 3-O-methyl glucose competitively, and conversely, the uptake of the probe is decreased in the presence of glucose. Measurement of the initial uptake of the probe gave values for K_t and V_{max} similar to those obtained for glucose in identical conditions, suggesting that both sugars are being transported by the same system. When intact adipocytes were treated with the radiochemically-labelled derivative, on photolysis only four hot membrane components could be identified in SDS polyacrylamide gels of the purified plasma membrane fraction. When homogenised material was used most of the membrane proteins become labelled. Two of the specifically labelled components were found to be glycoproteins with apparent molecular weights 100,000 and 81,000. In the presence of glucose, labelling of these components was significantly reduced, suggesting their involvement in the glucose transport system. Two lower molecular weight proteins, located on the external surface of the adipocyte membrane were also modified, but substrate protection could not be demonstrated.

The participation of membrane phosphorylation in the regulation of permeability has been implicated in, for example, toad bladder and rat brain synaptic membranes and two reports suggest that this process may be involved in modulating the effect of insulin on glucose uptake. Cuatrecasas (*J. biol. Chem.* **249**, 3170; 1974; **249**, 6854; 1974) reported that low concentrations of exogenous ATP can inhibit insulin-stimulated glucose transport and that this effect is associated with the phosphorylation of two membrane components of molecular weights 22,000 and 16,000 which can be separated from unlabelled components by SDS polyacrylamide gel electrophoresis. Phloretin, a strong competitive inhibitor of glucose transport, can pro-

tect cells from the ATP effect with concomitant reduction in the phosphorylation of both these components. In these experiments the binding of ^{125}I -labelled insulin was unaffected, as was the anti-lipolytic effect of the hormone. In further studies, it was found that in the presence of cyclic AMP, but not cyclic GMP, the same membrane proteins were rapidly phosphorylated, and a Ca^{2+} -sensitive cyclic-AMP dependent kinase was isolated from purified adipocyte plasma membranes. This system could not be found in guinea pig adipocytes, where glucose transport is not sensitive to insulin. The physiological importance of these findings is difficult to assess, particularly as no direct effects of insulin or adrenaline could be obtained by pretreatment of the cells with hormone before purification of the membranes.

However, more recently Avruch *et al.* (*J. biol. Chem.* **251**, 1511; 1976) have shown a significant increase in the phosphorylation of a 26,000 molecular weight component of adipocyte plasma membrane, in the presence of adrenaline (even at physiological concentrations) and it seems that this effect is reversed when insulin is also present. The relation, if any, of this protein to that described by Cuatrecasas is obscure. Higher molecular weight membrane components (79,000 and 62,000) could also be phosphorylated, but the extent of this was not hormone sensitive.

The development of the aryl azide derivative of glucose, which seems to be a potential probe for the facilitated glucose transport system provides an obvious strategy for the next step, determination of whether these phosphorylated proteins are involved in the regulation of glucose uptake. □

Plugging the gap in the astronomical spectrum

from a Correspondent

THE last gap in the electromagnetic spectrum available for observation by astronomers seems likely to be plugged in the near future, with the development of instruments purpose built for operating in the 1 to 10 mm band, between infrared and radio astronomy. One such instrument, with a dish 10 m across, has been proposed by observers at CalTech, where a small test dish has already been built. At the meeting of the Royal Astronomical Society on 11 March R. E. Hills, of the Mullard

Radio Astronomy Observatory in Cambridge described plans for a proposed UK instrument, with a dish 15 m across and designed to operate down to 0.8 mm.

This project—at least as a design exercise—got off the ground 18 months ago when the Science Research Council set up a steering committee, chaired by Anthony Hewish and managed by members of the Appleton Laboratory, with Hills as project scientist. The product now available is a fairly advanced design and specification for an instrument intermediate between a conventional optical telescope and a radio dish, requiring its own technology but incorporating ideas from both those areas.

Hills explained that the lack of observations in the millimetre gap has been due to inadequate technology; the photons in this band each carry too little energy to trigger the kind of detector used in, say, X-ray work, but the wavelengths are small enough to make observation by standard radio techniques difficult. With infrared observers pushing one way—stretching their instruments beyond their original design capability—and radio astronomers pushing the other, the gap has shrunk, and now the technology is available for purpose built instruments to complete the job.

Why should astronomers be so keen to plug this gap, when already today they have access to far more information, across a broader band of the spectrum, than ever before? Primarily, the prospects are exciting because this band corresponds to emission from cold material, at about 10 K, and therefore provides a window to view the very substantial fraction of matter in the Universe that is now known to exist in cold clouds. Star formation regions, cool envelopes surrounding old, evolved stars, the distribution of cold matter across individual galaxies, and the differences between galaxies, could be probed with the new telescope and there is also the prospect of penetrating deeper, in observational terms, into the dusty, active regions at the centre of many radio galaxies and similar objects.

How is this to be achieved? The instrument described by Hills, although larger than any existing optical telescope, would still be housed in a dome, but a dome with a rather large aperture in order for the large dish to have an unobstructed view. This would raise problems with wind blowing into the dome, which might be overcome by providing a screen of material, transparent at millimetre wavelengths, across the 'slit'—something suitable might be sailcloth backed with polythene. The instrument itself, the steer-

ing committee suggests, should be parabolic, designed on the homology principal which has proved so successful with some very large radio telescopes. This technique allows the instrument to bend under its own weight as it is moved into different orientations, but the design ensures that it always bends into a paraboloid; the effect is to shift the focus of the instrument, but it is much simpler to move the detectors sited at the focus as necessary than to attempt to keep the whole dish rigid.

Such a 15-m instrument should give 13 arc s resolution (better than single radio dishes; worse than optical telescopes) and would need a pointing accuracy of 2 arc s to make best value of the resolving power. The estimated cost of such a facility is £3.5 million, enough to make the SRC blanch at the present time, no doubt, but not so

great as to make this an obviously 'pie in the sky' project. The instrument would, of course, have to be built at a high site free from obscuring water vapour—the criteria are essentially the same as those which make Hawaii the site of the UK infrared telescope—and this would certainly mean that it would be run as a national facility for use by many teams, as large optical telescopes are, and would not be the *raison d'être* of a single group, as has generally been the case with large radio observational facilities. There is no doubt that the need for the facility exists, since nothing else now being planned will observe below 3 mm, and since most other planned instruments are restricted because they are to be built inside fixed radomes, with no opening slit. But any project requiring millions of pounds of new money today must have only slender prospects of realisation.

Pluto: the little-known planet

from David W. Hughes

PLUTO, the ninth and most distant planet in the Solar System has been and still is an object of considerable mystery and controversy. In 1930, when it was discovered, people were worrying about what to call it, Minerva and Pluto leading the field. Pluto, the brother of Jupiter and Neptune, won. Today, 47 years later, controversy still rages over its most fundamental physical parameters, its diameter, mass, density, surface covering and spin axis direction.

The first measurement of Pluto's diameter was made by Kuiper in 1950. Observing with the Hale Observatories 200-inch telescope at Mount Palomar during a period of excellent seeing, Kuiper compared the planet's image with that produced by a "disk meter", a device which produces an artificial image of adjustable brightness, colour and diameter. He obtained an angular diameter of 0.23 s of arc equivalent to a planetary diameter of 5,900 km. Unfortunately the systematic errors in such measurements of marginally resolved images are rather large. The fact however that Pluto did not occult a specific star in 1965 gave an upper limit to the diameter of 6,800 km.

An indirect approach to the diameter can be made by measuring the brightness of the planet and the albedo (or reflectivity) of its surface. Cruikshank, Pilcher and Morrison of the Institute for Astronomy, University of Hawaii, used the 158-inch Mayall tele-

scope at Kitt Peak National Observatory to measure the reflectance of Pluto in the near infrared (1.2 to 2.2 μm). They used standard JHK filters and two specially made narrow band filters centred at 1.55 and 1.73 μm . Their results are reported in *Science* (194, 835; 1976).

The most likely constituents of the surface of the outer planets of the Solar System are the ices or frosts of water, carbon dioxide, ammonia and methane. Water ice has already been found in the rings of Saturn, on four of Saturn's satellites and also on the Jovian satellites Europa and Ganymede. Fortunately reflectance measurements between 1.4 and 1.9 μm can distinguish between CH_4 , H_2O and NH_3 and Cruikshank *et al.* find that the ratio between the flux at a wavelength of 1.55 μm and that of 1.73 μm is especially useful diagnostically. For Pluto this ratio is 1.6 ± 0.1 which they interpret as indicating that CH_4 frost is the dominant reflecting material on Pluto's surface. The observations rule out the existence of large quantities of H_2O or NH_3 frost and of CH_4 hydrates. The CH_4 frost is probably mixed in with silicate or carbonaceous material.

There are two possible sources for this solid methane. First the temperature of the pre-planetary nebula could have dropped below 40 K at Pluto's distance from the Sun leading to the direct condensation of solid CH_4 . Standard equilibrium condensation

models would then indicate that Pluto has a mean density of 1.2 g cm^{-3} . Second, CH_4 8 H_2O could have condensed out at nearer 70 K, internal heating in the planet leading to the outgassing of CH_4 which subsequently froze on reaching the surface.

The brightness of a planet is proportional to d^2a/R^2 where d is the diameter, a the albedo and R the distance between the planet and the observer. Rocky planets and satellites (Mercury, Mars, Moon) have low albedoes (0.056, 0.16, 0.067), frost covered satellites (Europa, Ganymede, Rhea, Dione and Tethys) have albedoes in the range 0.4 to 0.6. The presence of CH_4 frost on Pluto would put its albedo into this range too. Assuming that Pluto has an albedo of 0.4 and a mean opposition magnitude of 14.90, the diameter would be 3,300 km. An albedo of 0.6 implies a diameter of only 2,800 km. Both these values make Pluto smaller than our Moon (diameter 3,476 km).

But Pluto is not completely covered with CH_4 frost. If it were its brightness would be constant. Between 1952 and 1955 the magnitude varied asymmetrically by about 0.11 with a period of 6 d 9 h 17 min, this being the rotation period of the planet. The brightness fluctuations are caused by successive passages through the meridian of regions of differing reflectivity. Andersson and Fix (*Icarus* 20, 279; 1973) found from observations made between 1971 and 1973, that the amplitude variation is now larger (0.22 mag) and the mean magnitude fainter (15.1). This is thought to be due to a change in the aspect of the planet as it moves round its orbit. The equatorial plane of Pluto is probably inclined at over 50% to orbital plane and the decrease in brightness is thus due to the sub-Earth point on the planet moving towards the equator during the past 40 yr, the polar regions having a higher albedo than the equatorial regions.

The mass of Pluto has been obtained from observations of the perturbations that this mass induces in the orbital motions of Uranus and Neptune. Seidelmann *et al.* (*Astr. J.* 76, 488; 1971) obtained, for example, a value of 0.11 ± 0.02 Earth masses ($6.6 \times 10^{26} \text{ g}$). However Ash, Shapiro and Smith (*Science* 174, 551; 1971) found that the value obtained for the mass of Pluto is extremely sensitive to the choice of perturbation data and parameter sets and that this mass cannot be determined with any certainty from this source.

If the density and size of the planet are known the mass can be easily estimated. The surface of Pluto inferred from the observations of Cruikshank *et al.* is exactly that to be expected for a large object which condensed at low

temperatures in the outer solar nebula. Its composition is probably dominated by frozen volatiles, just like most outer planetary satellites. (Remember there is a distinct probability that Pluto used to be a satellite of Neptune, but escaped during some encounter with Triton in the early times of the Solar System). If this is so its mean density is likely to be between 1 and 2 g cm^{-3} . Combining these with Cruikshank *et al.*'s values for the diameter (3,300 km if $a=0.4$, 2,800 km if $a=0.6$) gives mass values in the range 1.2×10^{25} to $3.8 \times 10^{25} \text{ g}$, about 1/250 the mass of Earth. This mass is much too small to perturb Uranus and Neptune in a measurable fashion, a conclusion which leads to two interesting consequences. First Clyde Tombaugh's discovery of Pluto must have been to a great extent fortuitous, the result of a diligent search and not an accurate, mathematically-derived, prediction as to the planet's position. Second if Pluto is not responsible for the perturbation of the orbits of Uranus and Neptune, something else must be; another planet or the cometary cloud perhaps? □

Palynology at Lucknow

from Peter D. Moore

The 4th International Palynological Conference was held at Lucknow on 29 December 1976–5 January 1977. The Proceedings are to be published.

OF particular interest at the conference were the accounts given of recent vegetation history in the tropics and subtropics, based upon studies of sub-fossil pollen grains stratified in peats, lake sediment and salt lake deposits. In contrast to the temperate areas of the world, the lower latitudes have been neglected and their recent environmental history is consequently little known. The work of J. R. Flenley (University of Hull) in New Guinea and A. P. Kershaw (University of Canberra) in north-eastern Australia has shown that tropical rain forest has not had the long uninterrupted Quaternary history which was once imagined. On the Atherton Tableland of Queensland, Australia, at an altitude of over 700 m, Kershaw has shown that the vegetation changed gradually from a microphyll vine forest to sclerophyllous woodland at about 25,700 b.p., which could have been due to increasing aridity or to the influence of early man, using fire. The development of rain

forest at the site did not occur until about 7,000 b.p.

Within India itself, the work of G. Singh (University of Canberra) and others has been of considerable value in elucidating the history of the deserts of western Rajasthan. The study of dry salt lakes in this area has shown that between 10,000 and 4,000 b.p. the sites were occupied by freshwater lakes and the pollen contained in these sediments demonstrates a development through *Artemisia* steppe to savannah vegetation with trees and shrubs. Desert encroachment followed this phase and is considered to be the product of climatic rather than cultural changes.

The argument about human involvement in recent vegetational changes is never easy to resolve. The presence of cereal pollen is often taken as evidence of human settlement and cultivation and therefore human potential for habitat modification. Cereal type pollen grains (40–50 μm) were found in the Rajasthan desert as far back as 9,300 b.p. and V. Mittre (Lucknow) reported even earlier finds of large Gramineae pollen in India. The Middle East records of cereal pollen were collated by A. Leroi Gourham (University of Paris); in Iran cereal pollen has been found in deposits going as far back as 60,000 b.p., thus demonstrating the existence of, presumably, the wild progenitors throughout the last glacial climatic episode. In Lebanon and Syria, herbaceous pollen was low during the forested times of the last glacial, but increased with forest decline (here cereal pollen is defined as being in excess of 60 μm).

The recognition of true cereal pollen, however, is not a matter over which all palynologists agree. Size criteria are most frequently used, but it is well known that mounting media can alter the sizes of pollen grains, they may swell in the course of time, and also some wild grasses have large pollen grains. E. Kohler and E. Lange presented some preliminary data on scanning electron microscope studies of grass pollen in an attempt to define cereals on more reliable morphological grounds. Only four genera (12 spp.) have been examined in detail so far, but four distinct types of sculpture have emerged. Sculpture alone however, does not provide a means of separating cereal from wild grass pollen. It is hoped that combinations of size, pore and sculpture type criteria will permit a more critical definition of pollen types within the Gramineae. Such a definition is vital to the furtherance of the study of primitive agriculture, its extents and its influence upon vegetation in the Middle East and India. □

review article

Modelling the structures of amorphous metals and alloys

J. L. Finney*

The application of random packing models to amorphous metals and metal-metalloid alloys is discussed, with particular reference to the effects of soft potentials, boundary conditions, and density. The local atomic organisations found leads to structural speculation concerning the glass transition, and the existence of 'amorphous polymorphs'.

SINCE the late 1950s when Duwez developed his 'splat-quenching' technique for the rapid cooling of liquids¹, a range of non-equilibrium, solid metallic alloy phases have been produced. Many such materials, and others produced by sputtering, and by electro-, chemical, and vapour deposition, exhibit interesting and potentially useful mechanical, magnetic and corrosion-resistant properties. More recently, amorphous (pure) metals have been prepared by vapour deposition on cold substrates²⁻⁴.

Many (though by no means all) of these materials are non-crystalline⁵. This non-crystallinity raises severe problems when we try to understand their structure-property relations. The absence of a regular underlying lattice organisation makes it difficult (and often dangerous) to apply many of the powerful methods of solid-state physics to such systems. Their structural characterisation is related more to dense liquids than crystalline solids. Rapidly quenched liquids at least might be directly related to the liquid organisation from which the 'glassy' solid is formed; thus there is little reason even to expect crystal nucleation to have taken place.

Use of models

This conceptual and mathematical vacuum—the absence of mathematical formalisms capable of dealing adequately with dense, non-crystalline aggregates of atoms or molecules—has been partly filled by the development of methods of structural modelling. Models of particular non-crystalline atomic or molecular aggregates can be built both in the laboratory⁶⁻¹⁴, and on a computer¹⁵⁻²⁸. Properties predicted by such (numerical) models can be compared with those observed experimentally, and disagreements used to improve the original model. Such modelling techniques were used extensively in the early days of crystallography. The question was asked: 'what structural arrangement of molecules can I construct which is (1) consistent with what I know about the way these molecules interact, and (2) consistent with the existence of a regular, repeating lattice in three dimensions?' Thus, the crystal chemist explained the structures of the rare gas solids in terms of close-packing of hard spheres, or ionic structures in terms of charge and size effects, and of diamond in terms of tetrahedral covalent bonding. Such explanations began as 'ideal' structures, which were later improved by taking into account more subtle effects such as the detailed nature of the potential function, polarisation and three-body interactions.

Modelling techniques as applied to non-crystalline solids are in principle similar. We ask a slightly different question: 'What structures can I build which are (1) consistent with the interactions between molecules (the 'potential function') but (2) not constrained by a regular lattice? As in the crystalline case, we establish first an 'idealised' structure of our amorphous metal or alloy. This

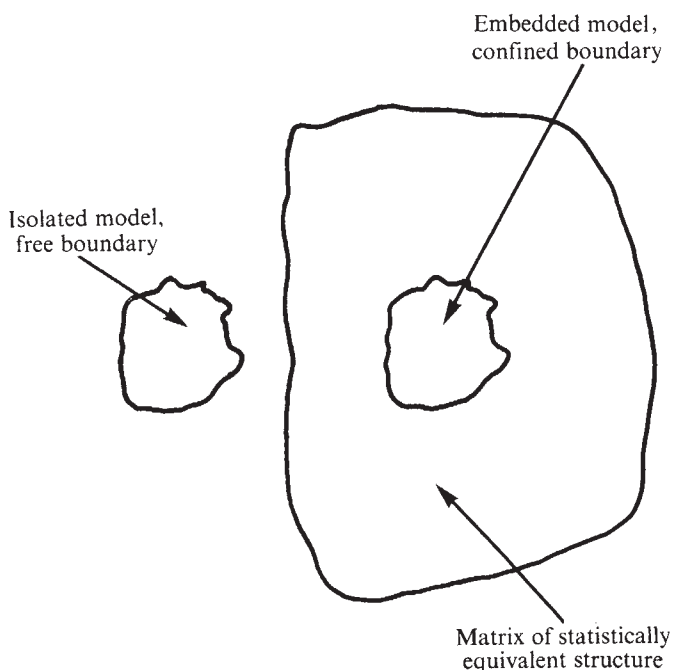
idealised structure should relate to the real structure as an idealised crystal structure (such as a cubic close-packing of hard spheres) relates to the real crystal. The idealised structure is then improved upon to explain in more detail the structure and properties of the real non-crystalline material.

Such modelling techniques have been applied specifically to a variety of non-crystalline solids, for example, silicate and other oxide glasses⁶⁻⁸, amorphous Si and Ge (refs 10, 26, 27), amorphous ice²⁹, and chalcogenide glasses³⁰⁻³². Although different systems present different problems to the modeller, the principles—and the pitfalls—are essentially similar in all cases. The structures of amorphous metal films (for example, Co, Ni (refs 2, 4) and glassy transition metal-metalloid alloys (NiP (ref. 5), CoP (ref. 28)) have been explored extensively by modelling and illustrate the principles and problems particularly well.

Specific application

To answer the model-builder's question above, we need first to strip the potential function down to those aspects which we think are structure-determining, and second to devise a method of

Fig. 1 The embedding problem. The structure of our finite model must correspond to that structure which would be consistent with its embedding in a matrix of (statistically) equivalent structure.



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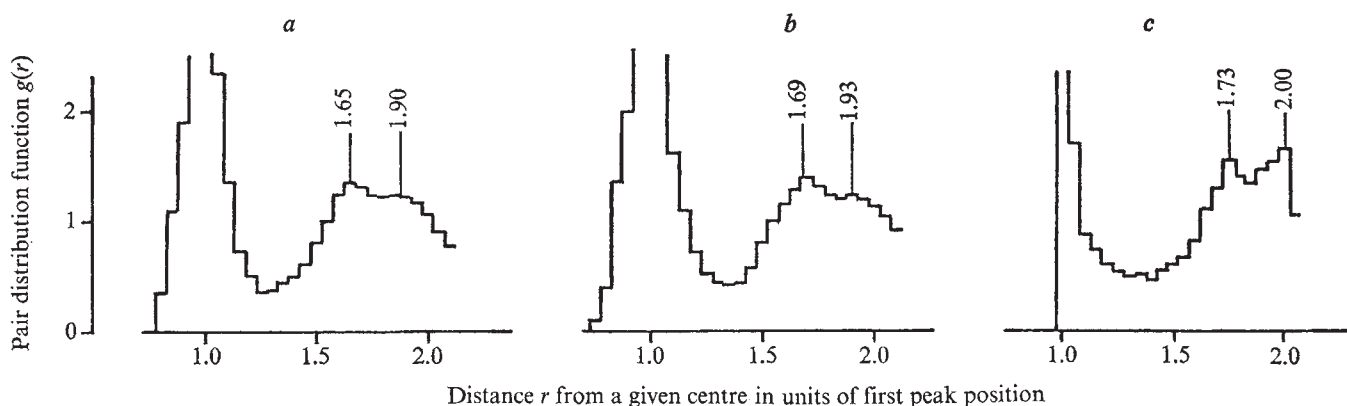


Fig. 2 Pair distribution functions of *a*, an amorphous bulk $\text{Ni}_{76}\text{P}_{24}$ alloy⁵; *b*, vapour-deposited amorphous Co (ref. 2); *c*, a random close-packed assembly of hard spheres¹⁴. $g(r)$ is the probability of finding an atom at distance r from any atom. The packing density of (*c*)—the ratio of total volume occupied to the total volume of spheres—is 0.6366 ± 0.0004 (ref. 14). The relative positions (r_2/r_1 and r_3/r_1) of the two components of the split-second peak are indicated.

building an array of a large number of such simplified molecules, consistent with what we otherwise know of the real system. In the metallic case, we simplify our atoms initially to hard spheres, expecting the structure to be determined largely by the repulsive core of the real potential function, as is known to be the case in crystals and liquids^{12,33–35}. However, when we try to put our idealised atoms together into a non-crystalline dense assembly, we run into difficulties which we must examine.

Boundary problems

Consider for example a hypothetical bulk amorphous metal. Any model we build can represent no more than a small sample of the bulk material. However, the model must be capable of extension indefinitely in three dimensions, without any significant change in structure. This gives rise to what might be called the 'embedding problem' (Fig 1). An adequate model must be consistent with its infinite extension; the model boundary must therefore be consistent with the constraints that would be imposed by the presence of similar arrangements of molecules at that boundary. This is particularly relevant when we consider the variety of methods used to prepare amorphous metallic assemblies. The growth process and the boundary conditions operative during the sequential deposition from the vapour of a thin film of amorphous cobalt are very different from those relevant to the production of an amorphous alloy by rapid quenching of the liquid. *A priori* there is no reason to assume identical structures will occur in these two extreme cases. A multiplicity of different amorphous structures may well exist, depending on the material and how it is prepared. Our modelling procedures should be capable of handling these possibilities.

Experimental comparisons

A general problem of characterising the structures of non-crystalline materials concerns the relatively low information content of the usual structural measurements. X-ray and neutron scattering from crystals can give a relatively vast amount of very specific spatial and dynamic information. In non-crystalline materials the spatial information obtainable is limited to a statistical average projection of the structure on to one dimension. By carefully Fourier transforming the coherent scattered radiation we can obtain a pair distribution function (PDF) (see Fig. 3). This tells us the probability of finding an atom at a distance r from any given atom. It is the crystallographer's Patterson function projected on to one dimension. This PDF is a relatively insensitive structural measure but because it is easily accessible from both real materials and model coordinates, agreement of the model and experimental PDFs is an essential filter for postulated models. Agreement is a necessary, but far from sufficient condition which a successful model must fulfil. Because of the series termination problems inherent in the Fourier transformation of the scattering

function³⁶, it is often preferable to compare real scattering functions with those predicted by a given model, especially when considering refined models. However, the structural reasons for disagreements between model and experimental scattering functions are not obvious in the scattering functions themselves. Thus, in what follows, we will use the PDF filter to examine postulated models and refinements, together with the densities of model and real material. This somewhat old-fashioned property is often easily measured, and provides an additional powerful filter which leads to rejection of many postulated models. The relatively high densities of amorphous metals and alloys are, in fact, not easily reproduced in model structures, because of the imperfectly understood packing restrictions in non-crystalline dense assemblies.

Early models

The first model to be widely applied to amorphous metals and alloys was constructed in the laboratory by packing together as densely as possible a large number of equal steel spheres, taking precautions to prevent ordering effect at the boundaries¹⁴. This work was initiated by Bernal in the late 1950s (refs 12, 13) to try to elucidate the geometrical nature of the liquid state. Similar work was performed independently by Scott in Toronto¹⁴. Cohen and Turnbull suggested³⁷ such models might be applicable to the then still hypothetical amorphous metal. However, its first specific application was to electrodeposited $\text{Ni}_{76}\text{P}_{24}$, whose X-ray scattering could not be reconciled with any realistic model based upon microcrystalline units⁵.

Fig. 2 compares the PDFs of electrodeposited $\text{Ni}_{76}\text{P}_{24}$, vapour-deposited amorphous Co (refs 2, 3), and the model dense hard-sphere packing out to two sphere diameters. Qualitatively, the agreement of both real systems with the model is good. The second peak is split in all three cases; at the time the hard-sphere model alone was able to account for this peak splitting. Moreover, the densities of model and experiment are of the same order, although precise quantitative comparisons are difficult as there is a degree of freedom in scaling the model to the real assemblies.

However, the quantitative agreement is far from perfect. The relative intensities of the two components of the split-second peak are predicted the wrong way round, and their relative positions r_2/r_1 and r_3/r_1 (measured with respect to the position of the 1st peak r_1) are slightly discrepant (Fig. 2). Moreover, how justified are we in applying such a simple single component model to both (1) a binary alloy stable in the bulk, and containing a slightly smaller atom of different chemical nature (the 'glass former' phosphorus), and (2) a thin film of material containing only one metallic component, and produced by sequential vapour deposition? We cannot really ignore the effect of a glass former, which may induce specific local structuring effects in bulk NiP case, even though the effect on the PDF may be small. Nor should we necessarily expect the film material, prepared by sequential

deposition from vapour, to have a structure similar to that of a model prepared to simulate specifically a bulk system.

Nevertheless, this agreement of PDF and density was sufficiently good to encourage proceeding on the postulate that the idealised hard sphere model incorporated the essential geometry of both types of system. We can now consider possible improvements to this idealised structure by examining the effects on it of, for example, different building methods, binary and more complex mixtures, different potential functions, and variations in boundary conditions. We are thus able to relate the above idealised structure more closely to that of the real materials.

Model refinements and alternative structures

Building and measuring large physical models in the laboratory is a painstaking business, and involves several imperfectly-controllable variables. Transferring the model-building to a computer facilitates tighter control of these variables, and enables structural variations to be investigated relatively rapidly. Nevertheless, there are severe restrictions on computer modelling. For example, it is very difficult to program those collective rearrangements of molecules that are necessary in order to reproduce the high densities obtained in laboratory models and the real materials, especially if large models are required. On the other hand, subtle structural effects of different potential functions can be readily examined, as can the effects of boundaries. Molecular dynamics simulations can generate structures consistent with external thermodynamic constraints in a way that both 'static' laboratory and computer building cannot hope to do. Even so, the

long relaxation times of non-crystalline or glassy materials, compared with the elementary molecular dynamics time step, presently limit full simulation calculations to quenching rates much higher than those achieved in the laboratory; hence these methods can produce little more than configurationally arrested liquids. However, taking together these three building methods, we can tackle effectively many of the interesting static and dynamic problems of glasses and non-crystalline solids.

Laboratory models

Little further laboratory modelling of amorphous metals and alloys has been reported. A recent study of 60:40 binary mixtures of hard spheres, size ratio 4:5, by Scott and Koniuk (personal communication) does show a reversal in the split-peak intensities, and also a reduction of the r_2/r_1 ratio to 1.89, much closer to the real $\text{Ni}_{76}\text{P}_{24}$ value. The r_2/r_1 ratio, however, remains unchanged at about 1.73.

Computer algorithms

Various procedures have been used to build hard-sphere packings both in the bulk and as thin films. The most widely used is the sequential building method of Bennett¹⁵, where each sphere is added in turn to a previously-existing tetrahedral site. The choice of site can be varied. Bennett originally used both 'global', and 'local' criteria. In the former scheme, a sphere is added at that site closest to the centre of the existing cluster, while the 'local' criterion chooses that tetrahedral site nearest to the plane of the three

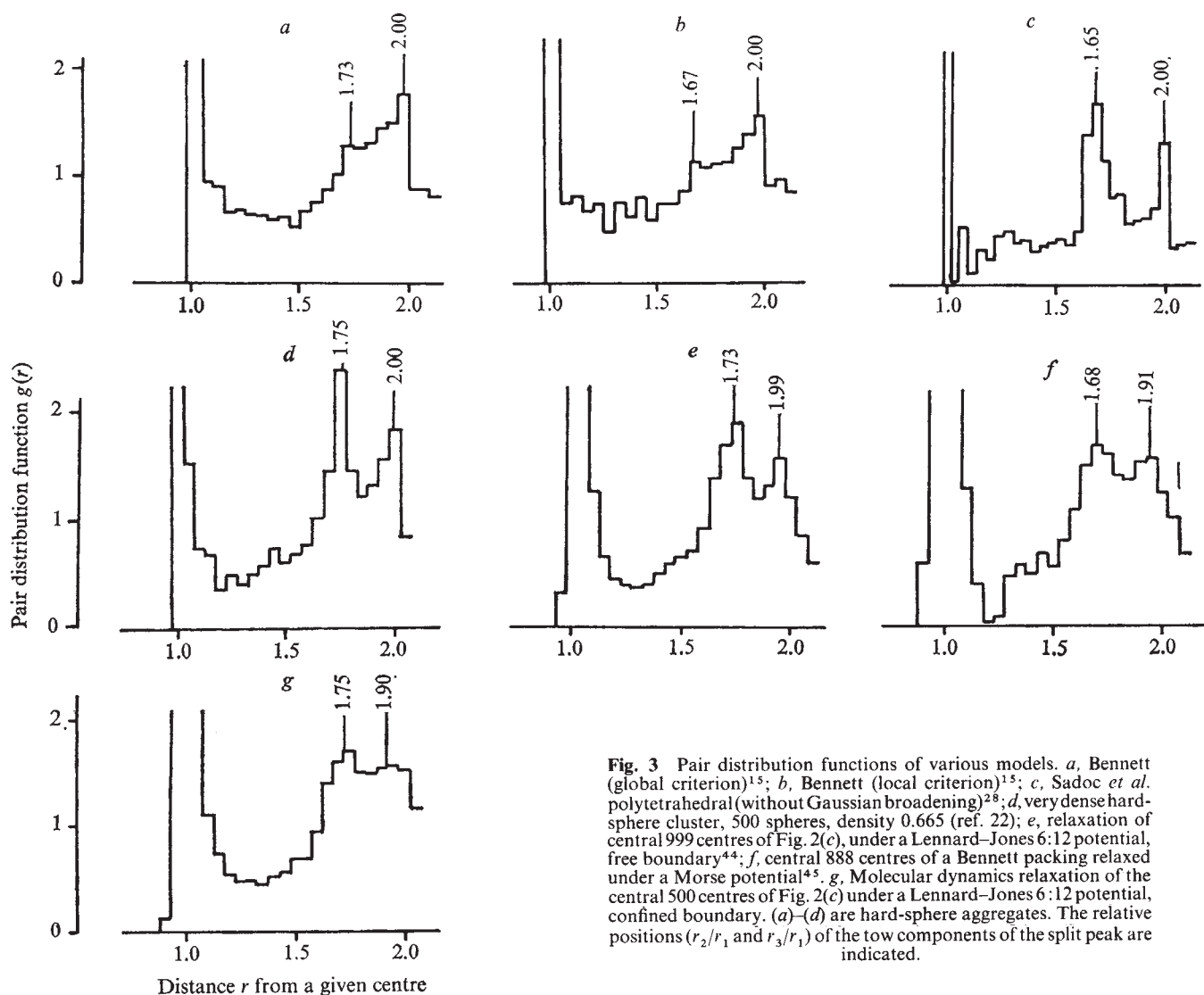


Fig. 3 Pair distribution functions of various models. *a*, Bennett (global criterion)¹⁵; *b*, Bennett (local criterion)¹⁵; *c*, Sadoc *et al.* polytetrahedral (without Gaussian broadening)²⁸; *d*, very dense hard-sphere cluster, 500 spheres, density 0.665 (ref. 22); *e*, relaxation of central 999 centres of Fig. 2(c), under a Lennard-Jones 6:12 potential, free boundary⁴⁴; *f*, central 888 centres of a Bennett packing relaxed under a Morse potential⁴⁵; *g*, Molecular dynamics relaxation of the central 500 centres of Fig. 2(c) under a Lennard-Jones 6:12 potential, confined boundary. (a)–(d) are hard-sphere aggregates. The relative positions (r_2/r_1 and r_3/r_1) of the two components of the split peak are indicated.

spheres defining it. Clusters of up to 4,000 spheres were built, and densities, extrapolated to infinite volume to eliminate size and boundary effects, were significantly lower (about 4%) than achieved in the laboratory packings. Sample PDFs are shown in Fig. 3(a and b). In both cases, the peak splitting is incomplete, reflecting the lower model densities. The peak positions of the 'global' model remain as in the laboratory packing, while for the 'local' model, r_2/r_1 alone is at 1.67 in better experimental agreement.

The Bennett procedure is very simple and fast, and has been used extensively with different boundary constraints, and with varying criteria for sphere additions^{15-21,25}. Several of these models reproduce r_2/r_1 relatively well, but fail to bring r_3/r_1 closer to experimental values. Moreover, and more seriously, the densities of such models are low. It seems that for hard spheres at least, reproducing the positions and relative intensities of the experimental split second peak components requires a model whose density is too low. The packing constraints in a sufficiently dense model seem to be inconsistent with the adequate presence of those local geometrical structures which are consistent with the experimental split-peak component intensities and positions. This density-local structure problem is illustrated further by the polytetrahedral model of Sadoc *et al.*²⁸. The algorithm used tries to reproduce the structural effects of the glass-former phosphorus in an amorphous Co-P alloy. In crystalline Co₃P each phosphorus is surrounded by 9 cobalt atoms in an incomplete icosahedral organisation. Sadoc's algorithm attempts to reproduce this icosahedral organisation, albeit slightly distorted from ideality. In Sadoc's resulting 'polytetrahedral' model, the relative peak heights are reversed, and the r_2/r_1 ratio is brought down to 1.65; r_3/r_1 remains at 2.00 (Fig. 3c).

However, this model also is not densely packed, having an average density 18% lower than random close packing³⁸. It can be considered as an aggregate of dense, distorted icosahedral units, with considerable void space between them³⁸. Thus, although the PDF of this inhomogeneous, clustered polytetrahedral model is qualitatively better than the homogeneous hard-sphere model, a consequence of forcing this clustering is, through the packing constraints, to prevent the achievement of a sufficiently dense assembly.

As long as we use hard spheres, the local structure which can explain the split second peak seems inconsistent with the high densities obtained in real materials. This is further underlined by an entirely different construction algorithm which simulates the compression of a hard sphere gas^{22,24}. Here, collective rearrange-

ments can be (rather laboriously) programmed, and very high densities achieved provided a free boundary is maintained. Figure 3(d) shows the PDF of such a 500-sphere cluster of packing density 0.664 (ref. 22). This is 4.2% higher than the 0.637 maximum obtained in the laboratory, a density which is thought to be the upper limit for a bulk random packing^{14,39-41}. Here again, although we can reverse the split-peak intensities, we cannot budge their position⁵. From an examination of the nature of local clusterings, an interesting result arises. As density increases, the proportion of distorted icosahedral clusters—Sadoc's polytetrahedral clusters—increases slowly, although constituting barely a few percent of the volume of the array²². Above a density of about 0.63, however, these 'spherically-polytetrahedral' units are forced out, demonstrating further that such locally dense clusters are indeed inconsistent with an overall high density.

This result is not surprising for hard spheres. The geometry of the icosahedron is such that the 12 surrounding spheres have some freedom of movement on the surface of the central one (Fig. 4). For soft spheres—more like the real molecules—the 'squashiness' of the atoms would be expected to stabilise considerably such local units (Hoare and McInness, personal communication). The question thus arises as to the interplay of increased icosahedral stability at the local level and the overall packing constraints in a real dense structure. Are the two tendencies more mutually compatible when the artificial infinitely-hard repulsive core is softened?

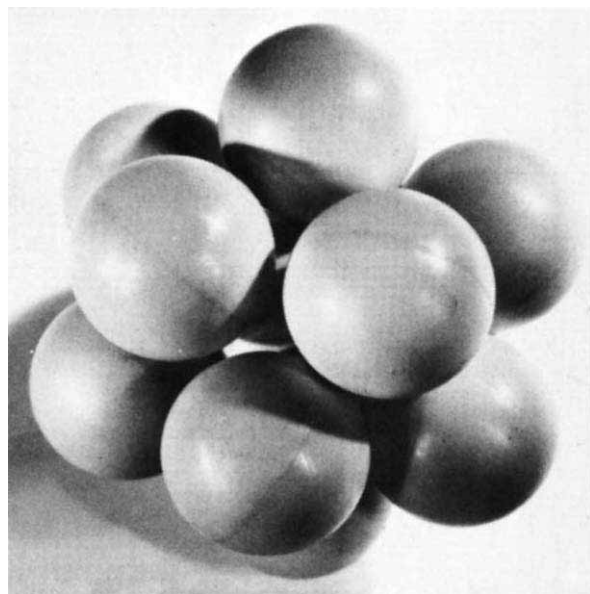
Computer refinements and molecular dynamics

The effects of softer potentials have been examined by (1) relaxing laboratory or computer-built hard-sphere models under various soft potentials⁴³⁻⁴⁶, and (2) very rapidly quenching liquid configurations by molecular dynamics^{41,47-50}. The results from both approaches are in excellent agreement, and give good second-order models directly related to the first-order idealised hard-sphere model. However, various questions about local geometry, and their relevance to the glass transition in metallic systems, remain to be answered fully^{38,51}. In one such relaxation calculation, the central 1,000 sphere coordinates in the laboratory-built hard-sphere model (Fig. 2c) were scaled to a Lennard Jones 6:12 potential and allowed to relax towards a potential energy minimum, in free boundary conditions⁴⁴. Movements were generally small, being limited by the high density of the starting configuration. Fig. 3e shows the relaxed PDF, the split-peak intensities again reversed, and only a small inward movement of r_3/r_1 was observed. r_2/r_1 was unchanged, and it was suggested from geometrical arguments that this peak position should be sensitive to the softness of the repulsive potential. The degree of local distorted icosahedral clustering increased, but only slightly. These results are in close agreement with recent simulated Lennard-Jones glasses produced by almost instantaneous liquid quenching^{41,48,49}.

Slightly different results were obtained in a series of molecular dynamics calculations with confined irregular boundaries using the same potential and starting configuration⁴⁶. Again an intensity reversal in the PDF was observed (Fig. 3g), and the inward movement of r_3/r_1 under the much higher pressure was significant. However, the degree of local 'spherical polytetrahedrality' was very low, such clusters having been presumably squeezed out under the very high pressure. Similar PDFs were obtained using a simulated harmonic potential²³; the local geometry was not examined.

The Lennard-Jones 6:12 potential is thought to have too hard a repulsive core for realistic metallic atoms. Hence a relaxation of a hard-sphere packing under a soft Morse potential (unfortunately again in free boundary conditions) is of particular interest⁴⁵. The PDF peak intensities reverse, and both r_3/r_1 , and r_2/r_1 fall to values closer to the experimental (Fig. 3f). Thus, the position of the first component of the split peak does indeed seem sensitive to the softness of the potential. Such a relaxed model gives relatively good PDF and density agreement for amorphous metallic films. The agreement with the binary metal-metalloid alloys is less good. The relation of the r_2/r_1 shift to the local cluster arrangement is as yet uninvestigated in such very soft sphere assemblies.

Fig. 4 Icosahedral arrangement of 13 spheres, related directly to the local geometry in 'spherically-polytetrahedral' models. This arrangement is that of minimum potential energy for soft spheres with a free boundary.



Conclusions

The work discussed above is, because of the widely varying conditions, and assumptions made, difficult to assemble into a coherent and water-tight picture^{52,53}. We can, however, make several generalisations about random packing, its application to real non-crystalline metallic alloys, and the problems and future prospects for modelling amorphous solids in general. We can also speculate a little about structural aspects of the glass transition and crystal nucleation.

We now have a much better idea of the nature of random packing. For hard spheres, a maximum packing density of about 0.637, is now well established for both modelling^{14,39} and molecular dynamics extrapolations^{40,41}. This maximum density can be achieved only by a relatively homogeneous assembly, with very little clustering into local icosahedral structures. Such clustering is prevented by the overall packing constraints. The homogeneous model is, however, polytetrahedral in that 70% of the assembly consists of relatively perfect tetrahedra¹³—naturally the densest local arrangement possible for four spheres. These tetrahedra are organised, however, not in the spherical clusters related to the stable icosahedral arrangements (Fig. 4) found in isolated atomic clusters^{38,42,51,54}, but in the twisted spirals of Bernal's 'pseudonuclei' (Fig. 5)¹³.

In adequately dense packings of slightly soft spheres, the tendency towards local 'spherically polytetrahedral' clusters (Fig. 4) increases, but only slightly⁴⁴. This increase is certainly very much less than that suggested in the much less dense specifically polytetrahedral models. 'Spiral-polytetrahedrality' (Fig. 5) is more stable under the local packing constraints found in dense assemblies. As long as the overall density remains high, there is insufficient volume available to allow the formation of many distorted icosahedra and their associated inter-cluster voids. It would seem that for molecules such as the rare gases where a Lennard-Jones 6:12 potential may be reasonable, the glass transition might more realistically be associated with the formation of relatively stable 'spirally polytetrahedral' clusters¹³ than the 'spherically polytetrahedral' ones stable in isolation^{38,42,51}.

For even softer potentials, the situation may be different. The extra freedom conferred by the softness of the potential may change the relative stability within a dense packing of spherical against spiral tetrahedral assemblies. A detailed geometrical analysis of such a relaxed assembly would help clarify this point.

The hard sphere packing provides an idealised model of the structure of several amorphous metal films and metal-metalloid alloys. All modifications made to achieve a better model result in improved intensities of the split second peak of the PDF (Fig. 3)^{22,23,43-45}. Only very soft potentials improve the position of the first component of the split peak (Fig. 3g)⁴⁵. The relaxed hard-sphere model is indistinguishable from a Lennard-Jones glass⁴⁸, while the hard-sphere model itself is indistinguishable from a hard-sphere glass as produced by molecular dynamics⁴¹.

The structural effects of boundary conditions can be significant^{38,44,46}. Thus we should not necessarily expect vapour-deposited materials to have the same structure as those produced by liquid quenching. Moreover, a splat-quenched structure and vapour-deposited structure may well not be mutually accessible directly. This underlines the possibility of 'amorphous polymorphs' of the same substance, and the possibility of different amorphous structure for different substances³⁸. Thus it would be interesting if amorphous phases of the same material could be produced by different routes, and their structural differences examined in detail, together with the possible routes for transformation between them^{51,52}. The clustering effects of glass-formers are likely to be significant though they are not well worked out.

Future modelling work on amorphous assemblies of spherical atoms must take adequate account of both the local effects of the potential functions and the overall packing constraints as reflected in the density and the boundary conditions. Structure can be very sensitive to both these factors. As a filter of possible model structures, agreement with the PDF is a necessary though far from

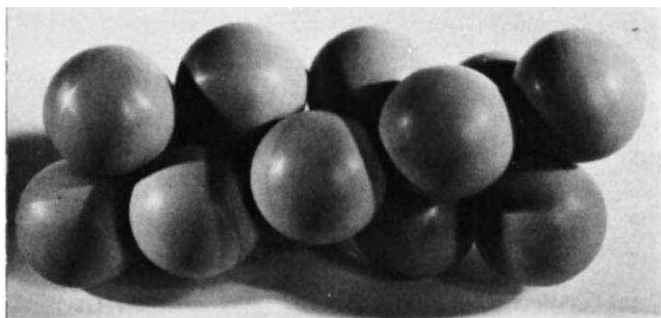


Fig. 5 A 'spirally-polytetrahedral' arrangement of 13 spheres, the so-called Boerdijk spiral of face-sharing tetrahedra. Such arrangements occur frequently in random packings of hard spheres.

sufficient condition. More attention should be paid to the old-fashioned property of density—qualitative agreement with PDF is hardly convincing if the density is unrealistically low.

The necessity to achieve high density models—and structural consistency with a bulk structure—raises severe difficulties for sequential building methods. These are basically unable to account for the high densities of real amorphous metals and alloys. Full molecular dynamics calculations can help overcome this, although the method is restricted severely by real time limitations. Although it is relatively straightforward to simulate a very rapidly-quenched liquid, the computer time required to simulate a quench on a realistic laboratory time scale, or a vapour deposition, is presently prohibitive. Thus, the detailed structural nature of the glass transition is not directly accessible thereby. Progress might be made by combining molecular dynamics with energy minimisation techniques⁵², as has been done for the much more difficult but related protein folding problem^{55,56}. These comments apply in general to the modelling of other glassy systems. Modelling is, however, still an inadequate substitute for more analytical approaches to the problems of local correlations in very dense, disordered systems. Although the last fifteen years of modelling a variety of amorphous solids, from the metallic systems considered here to oxide and chalcogenide glasses, has drastically increased our understanding of various non-crystalline systems, the methods become rather cumbersome when looking at refinements of first-order models. Moreover, especially in chalcogenide and more complex metallic alloy systems, the constraints that should be applied to the model-building may be insufficiently well formulated for the resulting models to be realistic. The interplay between the predicted properties of a postulated model, comparison with the real material, and feed-back to improve the model can be exceedingly time-consuming, and perhaps inconclusive once the basic idealised structure has been established. As the real amorphous systems we are interested in become more complex, the more difficult it becomes to handle, by simple modelling, the possible variables and possible heterogeneity of the structure. In some ways it is too easy to build models. To do it realistically, to represent a bulk system with adequate density, PDF, and density fluctuations in ill-defined or complex mixed systems is very difficult. And rather than turning a handle *ad infinitum* to improve marginally our models of simple systems whose basic structure we understand, we should pay more attention to the absence of an underlying theoretical framework, an absence which made modelling necessary in the first place. Only when such an adequate analytical approach to very dense, non-crystalline systems has been established—without the problems attached to integral equation approaches to the liquid state—will a full understanding of the complexities of these amorphous structures, their dynamics, their glass transitions and nucleation process, be realised. Data from models will have a role in the development of such techniques⁵⁷.

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articles

New palaeomagnetic evidence fails to support rotation of western Newfoundland

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Palaeomagnetic results from Ordovician to late Precambrian rocks in western Newfoundland are inconsistent with Wegener's hypothesis that the island has rotated 30° anticlockwise. Previous evidence apparently supporting this hypothesis is shown to be inconclusive.

WEGENER¹ proposed that Newfoundland has rotated 30° anticlockwise away from Labrador, implying a nearby pivot. A palaeomagnetic test of this motion is, in principle, straightforward, provided that test sites are carefully chosen (Fig. 1). Even so, the resolution obtainable may be small or zero, being a function of both the rotation angle R and the angular distance θ between pivot and ancient pole. Figure 2 illustrates this for Newfoundland ($R=30^\circ$), where values of P ('resolution') approaching 30° are obtainable at Lower Palaeozoic sites suitable for testing possible Devonian or later rotations. Hence a near-optimum test is feasible because of the low latitude of ancient Newfoundland.

After an inconclusive application of this test^{2,3}, Black⁴ compared Palaeozoic rocks of matching age in western Newfoundland and mainland eastern Canada, concluding that his data uphold a 30° anticlockwise rotation of at least western Newfoundland during the middle to late Devonian. Black argued that this would explain observed regional structures in the north-eastern Appalachians. Except for differences in latitude, his widely quoted Cambrian and Devonian comparisons conform strikingly to the positive test in Fig. 1. Regional and local tectonics^{5,6} and seismic results from the Gulf of St Lawrence⁷ have been cited as evidence against this rotation. Further, Black's data were found inconclusive⁸, because (1) the Cambrian remanence

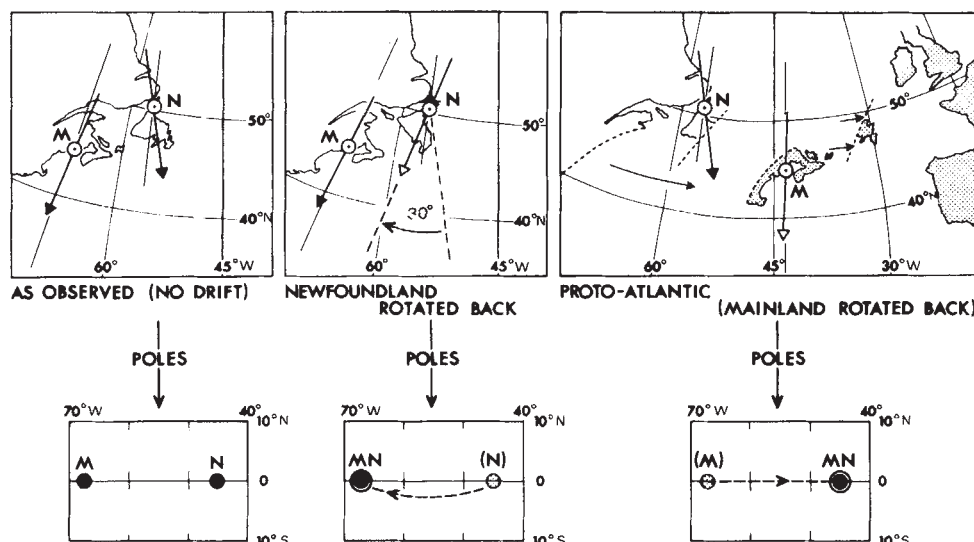
directions inferred in Newfoundland and New Brunswick (Fig. 3, S, R) are not significantly different; (2) the respective "Devonian" directions may not be contemporaneous; (3) by demagnetising only in alternating fields (AF) to 300 Oe, he was unlikely to have distinguished different high-stability components. Also⁹, since the mainland sites (Fig. 3, R) form part¹⁰ of the Avalon tectono-stratigraphic zone, rocks on opposite sides of the Central Mobile Belt were being compared (R, S). Therefore data in Fig. 1 could equally be taken to support either a rotation of Newfoundland or a proto-Atlantic model¹¹.

New magnetic data

To meet these objections we undertook new magnetic studies of mid-Ordovician to latest Precambrian rocks in Newfoundland (Fig. 3, N1–N3, N5–N7), using standard equipment. The results, summarised below and in Table 1, will be reported in greater detail elsewhere. Comparisons are with published data from tectonically stable mainland regions (Table 2).

Useful palaeomagnetic results have been obtained from carbonate rocks^{12–14}. We collected limestone of the Lower Ordovician^{15,16} St George Formation at 10 sites (62 samples) in the Port-au-Port area (N1). The natural remanence (NRM), carried in different samples by magnetite, haematite or both (compare ref. 14), was weak and fewer than half of the samples could be demagnetised. Haematite samples were unaffected by AF treatment (800 Oe) while thermal treatment was inconclusive, but in the magnetite samples both methods produced comparable, better-grouped new remanence directions (Table 1). St George limestone from Cape Norman (N2) gave similar results. This magnetic

Fig. 1 Palaeomagnetic test of rotation (schematic). Declinations of hypothetical 'observed' remanence vectors (solid arrows) and corresponding pole positions are shown for Newfoundland (N) and the adjacent mainland (M). Hollow arrows depict the restored vectors when either Newfoundland is rotated back¹ or the mainland site is rotated to a presumed location before the closing of a proto-Atlantic ocean¹¹. The pivots are at site N and 90°N, respectively. For convenience, poles have been located at the present equator. The two rotations here are indistinguishable⁹: an unambiguous test of the Newfoundland rotation requires comparison with a mainland site known not to have rotated. Stippled portions (right) correspond to the Atlantic faunal realm¹¹.



behaviour in Newfoundland limestones parallels that found earlier in the mid-Ordovician Trenton limestone¹² (M1), where it was shown that the stable component originated before the completion of diagenesis. This suggests a similar origin in the Port-au-Port limestone, but proof is difficult and it is uncertain whether one should correct for the gentle (12–15°) tilt which is associated¹⁰ with the Taconic (pre-mid-Ordovician) or Acadian (Devonian) orogeny or both. The (uncorrected) mean directions for areas N1 and N2 after AF or thermal treatment are all similar (Table 1). Pole N1 (Table 2) is based on sample rather than site statistics, since too few samples per site remained measurable after AF treatment. The tilt-corrected pole is 22°N, 120°E. Pole N1 is not significantly different from Pole N4 (ref. 13) for the St George Formation, where no tilt correction was reported.

A result¹⁷ from pillow basalt and sheeted dikes of the Moreton's Harbour Group (N3) is useful for comparison with the St George Formation, since different rock types of similar ages are being compared. Most or all of the 8-km thick Moreton's Harbour sequence has been attributed^{18,19} to island arc volcanism in the Lower¹⁹ to Middle¹⁸ Ordo-

vician. Although these rocks lie in the Central Mobile Belt (Fig. 3), it seemed reasonable to include them in a test based on western Newfoundland, since they probably originated as part of the North American margin (D. F. Strong, personal communication). A stable remanence in magnetite, isolated by AF demagnetisation, presumably was acquired not later than the generally low-grade greenschist metamorphism of these rocks. The tilt-corrected pole (Table 2) is not significantly different from poles N1 and N4 (Fig. 4).

Lower Cambrian¹⁵ Bradore sandstones sampled by Black⁴ at St John Bay (Fig. 3, S) were re-sampled more extensively²⁰ at Canada Bay (N5). The NRM, carried by haematite, had a mean direction as in Black's AF-treated samples (Table 1), but by thermal demagnetisation a stable component misaligned with both the NRM and Black's direction was isolated at 665 °C: this shows that the objection⁸ to exclusive low-AF treatment is well-founded. This component probably pre-dates the gentle (15–25°) Taconic and/or Acadian tilt of the sediments, though a post-tilting origin of the entire NRM is not impossible²⁰. The stable direction corresponds to a reverse pole at 23°N, 160°E

Table 1 New palaeomagnetic results from Newfoundland

Area no.	Rock unit/area	Geological age	N	(n)*	Mean NRM direction†				Mean direction after treatment†			
					Declination (°)	Inclination (°)	k	Treatment‡	Declination (°)	Inclination (°)	k	α_{95} (°)
N1	St George limestone, Port-au-Port area	Early Ordovician ¹⁵	5S§	(14s)	179.5	+62	63	300 Oe	181.5	+25	910	2.5
			15s§	—	179.5	+57.5	13	300 Oe	182	+23	90	4
			6s	—	159	+61.5	49	500 °C	183	+18.5	200	5
N2	St George limestone, Cape Norman area	Early Ordovician ^{15,16}	5s	—	164.5	+73	70	400 °C,	177	+22.5	120	7
N3	Moreton's Harbour basalt	Early to mid-Ordovician ^{18,19}	11S	(56s)¶	114	+66	8	150 Oe	(116.5	+57	9	16.5)
									176	+16	9	16
N5	Bradore sandstone, Canada Bay	Early Cambrian ¹⁵	9S	(54s)	153.5	+52	13	665 °C	146.5	+18	110	5
N6	Bradore sandstone, St John Bay	Early Cambrian ¹⁵	2S	(13s)	198	+66	—	665 °C	132.5	+25.5	—	—
S	Bradore sandstone, (Black's result) ⁴	Early Cambrian ¹⁵	10s	—	?	?	?	300 Oe	151	+44.5	27	9.5
N7	Cloud Mountain basalt	Latest Precambrian**	3F	(10S)	159.5	+73	14	100 Oe	(142	+50	840	4.5°)
									127	+36.5	1250	3.5°)

*N, number of flows (F), sites (S) or samples (s) used in averaging; (n) number of sites or samples studied.

†Angles rounded to 0.5°; k values greater than 100 are rounded to nearest 10; where two sets of data are given, those in brackets were obtained before correction for geological tilt and those without brackets are tilt-corrected and were used in Table 2. In all other cases, no tilt correction was made.

‡Stepwise thermal or AF demagnetisation, with fields quoted in peak oersteds.

§Site and sample statistics for the same samples are shown for comparison; samples (second row) are used in Table 2.

||Three samples were treated to 400 °C, two samples to 300 Oe.

¶Reduced to 46 samples (11 sites) after treatment.

**⁴⁰Ar/³⁹Ar date of 605 ± 10 Myr cited for Long Range dikes (ref. 25), which are considered (refs 24, 25) to be contemporaneous with Cloud Mountain basalts.

Table 2 Palaeomagnetic poles for Newfoundland and mainland North America

Area no.	Rock unit/area	Geological age*	N†	Treatment†	Latitude (°)	Pole position		Polarity†	Mean rotated pole‡	
						Longitude (°)	dp, dm (°)		Latitude (°)	Longitude (°)
N1	St George limestone, Port-au-Port area	Early Ordovician	15s	a	30 N	119 E	2,3	R	26 N	86 E
N3	Moreton's Harbour basalt	Early to mid-Ordovician	11S	a	32 N	130 E	9,17	R	27 N	96 E
N4	St George dolostone, Daniel's Harbour	Early Ordovician ^{15,13}	8S	a	29 N	125 E	3,7§	N	25 N	92 E
M1	Trenton, limestone, New York	Mid-Ordovician ¹²	29s	a	36 N	114 E	3,5	R		
M2	Front Range intrusives, Colorado	'Cambro-Ordovician' ²⁶	18s	a	37 N	122 E	6,12	R		
			14s	t	44 N	100 E	5,11	R		
			—	a + t	40 N	111 E	—	R		
N5	Bradore sandstone, Canada Bay	Early Cambrian ¹⁵	9S	t	23 N	160 E	3,5	R	30 N	128 E
N7	Cloud Mountain basalt	Latest Precambrian	3F	a	5 N	172 E	2,4	R	17 N	146 E
M3	Franklin intrusives, Northwest Territories	Latest Precambrian ^{29,31}	46S	a	6 N	166 E	4,4	M		
N8	Indian Head anorthosite	'Grenville' ³²	5S	a	8S	158 E	15,20	N	1 N	136 E
M4	Morin anorthosite, Quebec	'Grenville' ³³	13S	a	O	164 E	9,12	R		
M5	Northern Grenville anorthosite, Ontario	'Grenville' ³⁴	4S	a	8 N	161 E	6,6	R		
M6	Whitestone anorthosite, Ontario	'Grenville' ³⁵	8S	a	5 S	168 E	6,10	R		
M7	Charlevoix anorthosite, Quebec	'Grenville' ³⁶	5 S	a	7 S	148 E	12,16	R		

*Ages are discussed in the references (see also Table 1). Ref. 26: Radiometric ages in the range 601–427 Myr are cited. Ref. 29: The authors cite numerous K/Ar ages with 'best mean age' 650 Myr, but they adopt 675 Myr, to allow for argon loss. Ref. 31: Other Franklin igneous sites have K/Ar ages matching an isochron of 625 Myr. Refs 32–36: 'Grenville' ages in the range 0.9–1.0 × 10⁹ yr are cited, based on radiometric data or on inferred time of magnetisation.

†N, see footnote to Table 1; Treatment: a, alternating-field; t, thermal; a + t, mean of poles based on a and t treatment; Polarity: N, normal; R, reverse; M, mixed.

‡Average values are quoted for 30° clockwise rotations about pivots A (51.5°N, 56.5°W) and B (48°N, 59.5°W) (Fig. 3).

§Recalculated from original data.

||Error circles (α_{95}) instead of ovals are cited by authors.

(Table 2), or 29°N, 167°E after tilt correction. Bradore standstones from St John Bay (N6) gave comparable (virtual) poles.

The gently dipping Cloud Mountain plateau basalts (N7) were sampled at 10 sites. They have been correlated stratigraphically²¹ and through magnetic similarities^{22,23} with plateau basalts in south-east Labrador of presumed late Precambrian to early Cambrian age²¹. Strong and Williams²⁴ consider the Cloud Mountain basalts to be co-magmatic with and contemporaneous with the chemically similar Long Rang dikes, which have a ⁴⁰Ar/³⁹Ar dating of 605 ± 10 Myr (ref. 25). Only three separate flows could be identified. AF treatment isolated a stable component carried by magnetite, resulting in greatly improved within- and between-flow vector groupings. Its mean direction after tilt correction corresponds to the pole shown in Table 2. We note that a residual secular variation component may be present in these few flows.

Rotation test

There is no unique pivot for the postulated rotation of western Newfoundland. Black⁴ relocated his Newfoundland palaeomagnetic poles by rotating the island 30° clockwise separately about two extreme pivots A and B (Fig. 3) using the mean restored pole position to compare with poles on the mainland. A similar procedure is followed here (Table 2). The uncertainty in pole positions due to averaging about the two pivots is ≤ 1°.

Out of the three identical Ordovician Newfoundland poles, N1 and N3 are in turn identical with, and pole N4 is close to, the Trenton limestone pole¹² (M1). Here 'identical'

is defined non-rigorously by an overlap of the 95% confidence ovals (Fig. 4). Pole M2 from the Front Range Intrusives²⁶ of uncertain Cambrian to Ordovician age is shown without oval, being the average of two poles equally weighted in ref. 26, based on AF and thermal treatment, respectively; the former but not the latter pole is identical to poles N1, N3, N4 and M1. With Newfoundland rotated back, the 'N' and 'M' poles become discordant, except pole N3 for which the test is inconclusive. These results are

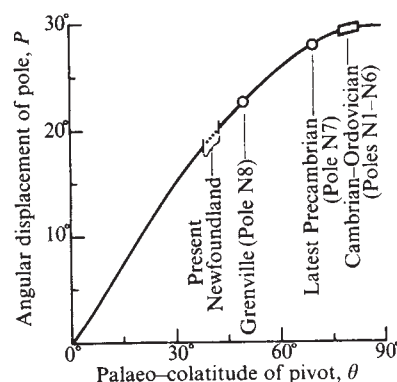
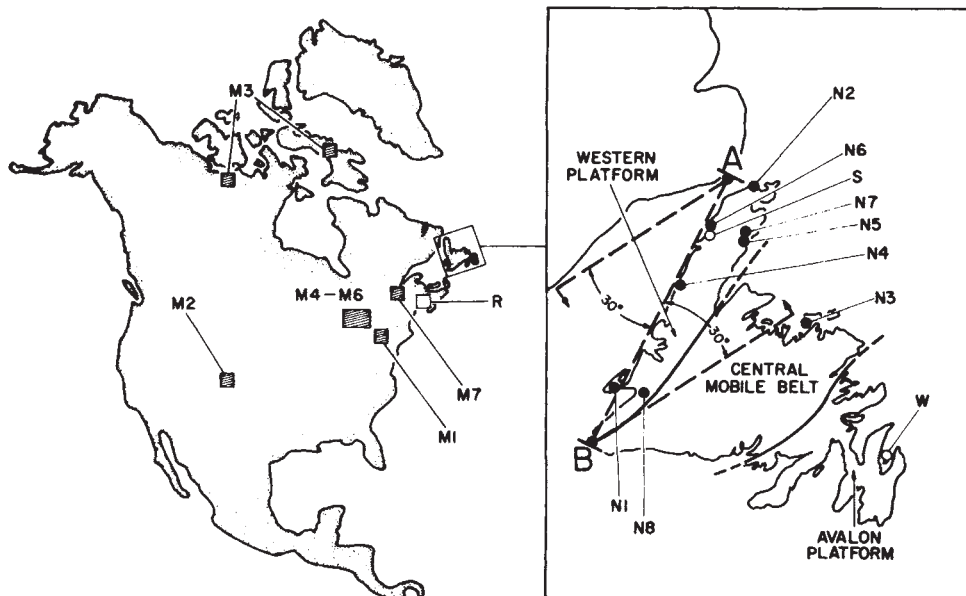


Fig. 2 Resolution (P) obtainable by palaeomagnetism for testing rotation (R) of a land mass, as a function of the angular distance (θ) between pivot and ancient pole, where: $1 - \cos P = \sin^2 \theta$ ($1 - \cos R$). P is the angular shift of the ancient pole on restoring the land mass to its pre-drift position. P cannot exceed R . Illustrated for the Newfoundland hypothesis ($R = 30^\circ$) using actual results (Table 2). Note that, with minor error in θ , site locations N1–N8 may be substituted for the average pivot locations A and B (Fig. 3).

Fig. 3 Palaeomagnetic sites for testing rotation of Newfoundland (Tables 1, 2). R, Ratcliffe Brook, New Brunswick (Lower Cambrian)⁴; M1-M7, other mainland sites. S, St John Bay⁴; W, Wabana³⁷; N1-N7, other Newfoundland sites. A, B, alternative pivots⁴ (Table 2).



incompatible with the proposed rotation, at least since mid-Ordovician time. A provisional Upper Ordovician pole (R. Van der Voo, personal communication) at 29°N , 118°E from thermally cleaned red sandstones of the Juniata Formation, Pennsylvania, is not significantly different from poles N1 (30°N , 119°E) N3, N4 and M1. This agreement may be due to insignificance of mid- to late-Ordovician polar motion relative to eastern North America, in which case the Juniata result is additional evidence against rotation of western Newfoundland.

Comparisons of the Bradore data²⁰ (N5) with those from Cambrian mainland localities that avoid the proto-Atlantic ambiguity are difficult, since the few reliable mainland poles (see ref. 27) have been cited as Upper rather than Lower Cambrian in age; also they are widely dispersed, suggesting rapid polar displacement. Hence a rotation test using the Bradore result seems premature. A Lower to Middle Cambrian pole at 13°N , 157°E for eastern Quebec was recently reported²⁸, however. Its fair agreement with the Bradore pole (23°N , 160°E) is destroyed when Newfoundland is rotated back. This may be taken as evidence against the proposed rotation.

On the mainland the youngest suitable Precambrian rocks are the Franklin intrusives²⁹⁻³¹. Of numerous K-Ar datings, 675 Myr is the adopted age³⁰ cited for pole M3 (Table 2), but other Franklin diabbases and lavas³¹ give a pole close to

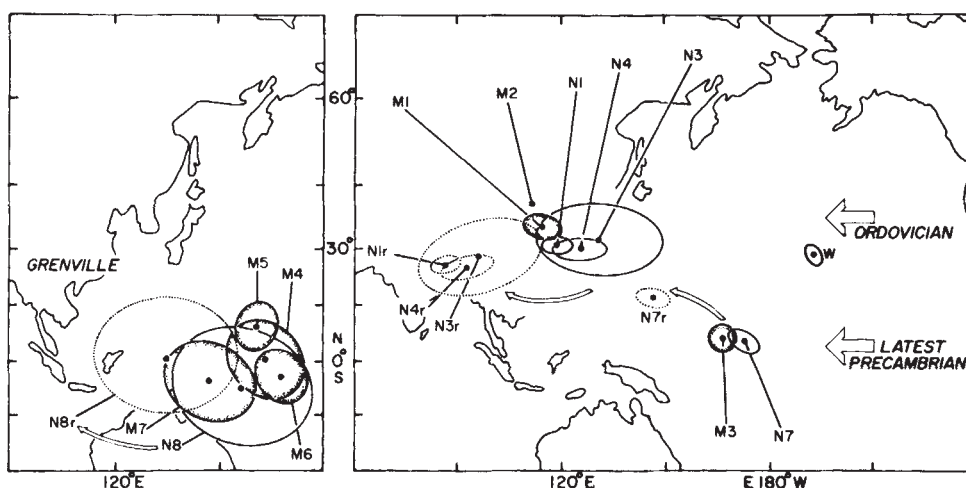
M3 and are dated 625 Myr, compared with the 605-Myr age inferred for Cloud Mountain (N7). Figure 4 shows agreement between poles N7 and M3, but they strikingly diverge when Newfoundland is rotated back. Pending better knowledge of the Newfoundland-mainland age difference and of the time span averaged in the Cloud Mountain lavas, this result again is inconsistent with the rotation hypothesis.

The minimum radiometric age of 890-900 Myr of the Indian Head anorthosites³² (N8) suggests that they may be contemporaneous with mainland rock formations dated at the time of the Grenville orogeny (1.0×10^9 yr). Pole N8 was compared with only mainland poles dated $0.9-1.0 \times 10^9$ yr, based on anorthosite (M4-M7)³³⁻³⁶, to make it more probable that a synchronous Grenville event is being tested. Once again an initial agreement between 'N' and 'M' poles is lessened when Newfoundland is rotated back (Fig. 4). Unrotated, the oval of pole N8 overlaps all mainland ovals, whereas after rotation it misses two of these (M5, M6). The large size of oval N8, however, makes this test only marginally significant.

Significance of the test

Newfoundland data from the Ordovician, latest Precambrian, more marginally the Grenville and perhaps also the Cambrian, when compared with the mainland results, provide evidence against Wegener's¹ hypothesis. At the

Fig. 4 Negative results of the rotation test for three age levels. Pole positions with 95% confidence ovals (Table 2) for the mainland (M) (shaded ovals), and Newfoundland (N) as observed (solid ovals) and after rotation (dotted ovals), site numbers followed by 'r'. W, Wabana (Fig. 3 and text)³⁷. Magnetic north or south poles are plotted to correspond to presumed north rotation poles.



same time we have verified that Black's⁴ Cambrian results used by him in support of that hypothesis are inconclusive. Generally better data were available to us than to the earlier workers^{2,4} in terms of statistics, magnetic control and comparative dating. Limitations are, however—(1) some large confidence ovals; (2) different populations (samples, sites, flows) being compared; (3) the small number of usable limestone samples (15) and of Cloud Mountain flows (three); (4) uncertainty regarding tilt corrections; (5) possible anachronisms between poles of any given age; and (6) the absence of a reliably Lower Cambrian mainland result to compare with pole N5.

Partly because of these limitations, we could not employ rigorous statistics. For example, an *F* test applied to negative evidence on rotation would ideally show, in each case with high probability, that the mainland and unrotated Newfoundland poles for a certain age are identical and that they become distinct when Newfoundland is rotated back. Comparing actual data, such as Ordovician poles N1, N4, M1, the latter condition is certainly met, but the former condition is only moderately probable. The difficulty is predictable from Fig. 4 and recurs in an *F* test of the Grenville poles. This restricts us to the use of confidence ovals for assessing significance.

We conclude that the evidence is inconsistent with western Newfoundland having rotated by 30° since the late Precambrian, and is consistent with the absence of any rotation, although a rotation by 5°–10° since the Ordovician is possible. To determine whether such lesser rotation actually took place calls for more refined palaeomagnetic data from Newfoundland and North America.

Finally, a Lower Ordovician pole from Wabana, eastern Newfoundland³⁷ (W, Figs 3, 4) is seen to be far displaced from poles N1 and N4 of similar age in western Newfoundland. This is compatible with the hypothesis¹¹ of a Lower Palaeozoic proto-Atlantic Ocean of undetermined width, in which the two portions of Newfoundland occupied opposite

coasts. Poles W, N1 and N4 may be made to coincide by rotating Wabana towards Europe about some pivot in the central Atlantic.

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Current rifting episode in north Iceland

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A major rifting episode is now occurring in north Iceland. This started on 20 December 1975, with a basaltic eruption, an exceptionally intense earthquake swarm and movement on an 80-km segment of the plate boundary. Inflation and deflation of the Krafla caldera indicate upwelling of magma and injection into the rift zone. Historical records show that similar episodic rifting occurs in this region every 100–150 yr.

The plate boundary between the American and European plates runs through Iceland as a zone of active volcanism, seismicity and graben structures. The two plates are drifting away from the boundary at a velocity of about 1 cm

yr⁻¹ (ref. 1), but the mechanism of this drifting process at the boundary itself has not been clearly observed until now. A spectacular rifting episode started in northern Iceland on 20 December 1975, and is still not completed in December 1976. Intense earthquakes, widespread ground deformation and magmatism are affecting an 80-km long segment of the plate boundary. We describe here the geological setting of these events, the main observations to date and an attempted interpretation.

In north Iceland the Neovolcanic Zone has a north-south direction. Towards the south it connects with the Eastern Volcanic Zone of south Iceland, but towards the north it is offset to the west by about 100 km, by the Tjörnes Fracture Zone, to join the Kolbeinsey Ridge (Fig. 1). The structure of the Neovolcanic Zone in north Iceland

is dominated by large swarms of faults and fissures, most of which pass through different central volcanoes where volcanic fissure eruptions, silicic rocks and high-temperature geothermal fields are concentrated². Two central volcanoes in this region, Krafla and Askja, have developed calderas. The fault and fissure swarms are arranged *en echelon* subparallel to the north-south direction of the main zone. The northernmost parts of these fissure swarms are intersected in the area of Axarfjörður by the Tjörnes Fracture Zone, which has an east-west direction.

The Tjörnes Fracture Zone is a zone of high seismic activity and large earthquakes, most of which seem to be associated with right-lateral strike-slip on WNW striking faults, one of which, the Húsavík fault, has been identified on land, and another, the Grímsey fault, inferred from earthquake location and a fault plane solution. These faults run parallel to each other and are about 40 km apart. The Tjörnes Fracture Zone cannot therefore be viewed as a simple transform fault²⁻⁴. The present events have been confined to the Krafla caldera, the associated fault swarm to the north and a segment of the Grímsey fault (Fig. 1).

Similar tectonic sequences have occurred before in the Neovolcanic Zone: the last eruption associated with major tectonic movements occurred in 1874–1875 in the Askja caldera and the fault swarm to the north. In 1724–1729 an eruption took place in the Krafla caldera and ground fissures moved in the associated fault swarm.

Attempts made since 1938 to measure ground deformation in this part of the volcanic zone, by measurement of horizontal movement have so far yielded ambiguous results^{5,6}. Measurements of vertical movements here since 1966 have shown subsidence of about 10 mm yr⁻¹ (ref. 7). Preliminary measurements show that horizontal and vertical ground movement during the present event is at least 2 m.

A geothermal power station (60 MW) is now under construction inside the Krafla caldera, a diatomite factory is located on the fault swarm to the south near Námafjall, and a small village is situated at Reykjahlíð (Fig. 2). A more intensive effort has therefore been made to investigate thoroughly all aspects of the present tectonic events.

Geology of the Krafla–Axarfjörður area

The present activity is taking place mainly within the Krafla caldera and in the Axarfjörður depression. The two areas lie on a fault swarm which is about 100 km long and has a maximum width of 10 km (Fig. 1).

Basaltic fissure volcanism is concentrated mainly within the Krafla caldera and near Námafjall (Fig. 2). In post-glacial time about 20 eruptions have occurred in the Krafla caldera and its nearest surroundings, and about 15 in the Námafjall area. Most of the fissure eruptions are basaltic, but andesite and dacite flows have also been erupted in both areas. Voluminous silicic eruptions have not occurred in postglacial time but four subglacial silicic eruptions within and around the Krafla caldera have produced large domes or ridges during the last glacial period. The Krafla caldera contains explosion craters that have ejected small quantities of rhyolitic pumice. The most recent crater formed in 1724 at the beginning of the 1724–29 volcanic episode (Myvatn fires).

Tephrochronological studies⁸ indicate that postglacial volcanism in the Námafjall and Krafla areas occurred in two main periods: in early postglacial time, and during the last 3,000 yr. The period of repose may have lasted more than 4,000 yr. The Krafla and Námafjall parts of the fissure swarm have erupted 6–7 times each during the latter period of activity. At least two of the eruptions are common to both areas. Volcanism near Krafla has been concentrated along the central part of the fissure swarm during this period; earlier, it was dispersed over the entire caldera. The largest volume of basaltic lava produced in a

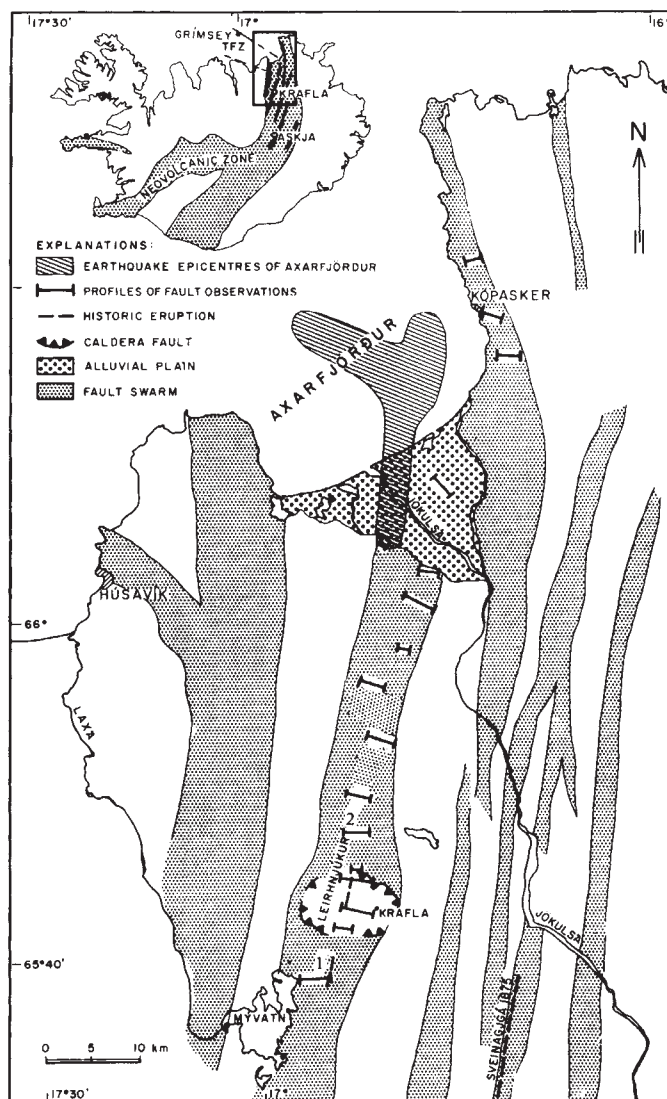


Fig. 1 Fissure swarms in the Neovolcanic Zone of north Iceland. The present rifting is taking place in the swarm through the Krafla caldera and extends from Myvatn to Axarfjörður. Inset map shows the location of the area in relation to the Neovolcanic Zone and the Tjörnes Fracture Zone (TFZ). Dates of activity: 1, 1727; 2, 1725–29, 1746, 1975.

single eruption in the area came from the Threnslaborgir–Lúdentborgir crater row on the Námafjall part of the swarm about 2,000 yr ago. Its total volume has been estimated as 2–3 km³ and the areal extent is about 220 km² (refs 9–11). Few flows of the swarm, however, exceed 0.2 km³.

The Krafla caldera measures about 10 km east–west and about 8 km north–south. It formed during the last interglacial period and has since been filled almost to the rim with volcanic material. The caldera's collapse almost certainly followed the eruption of a sheet of dacitic welded tuff which is exposed around the caldera (Fig. 2), and is an airfall tuff, blown mainly towards the north-east from a source near the centre of the caldera. A shield volcano with a diameter of at least 20 km existed before the caldera formation, and its remnants enclose the caldera on the east and west sides, exposing lavas and breccias dipping outward at low angles. Dykes and eruptive fissures trending parallel to the caldera ring fault are conspicuous around the caldera.

A high-temperature geothermal field lies within the caldera. Drilling has revealed temperatures >340 °C at

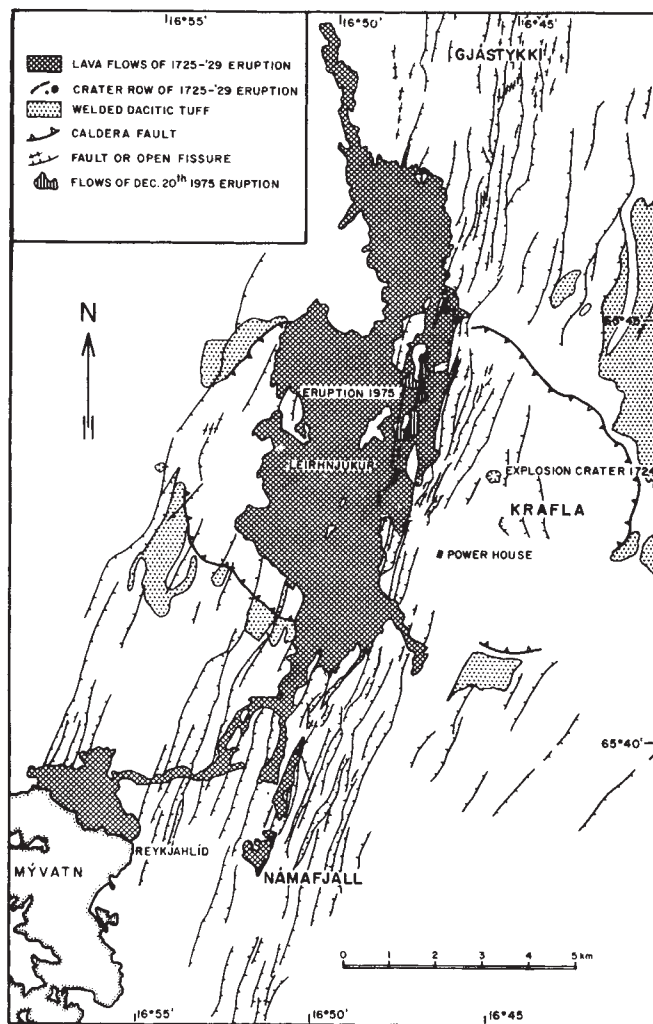


Fig. 2. Outline geological map of the Krafla caldera and the associated fault swarm.

2 km. Basaltic intrusions, with dolerite and granophyre, become increasingly abundant below 1.2 km. Corresponding coarse-grained rocks are conspicuous among xenoliths of the welded tuff and the ejecta of the 1724 explosive eruption. Another high-temperature geothermal field exists at Námafjall, and drilling to a depth of 1.8 km, revealed temperatures of over 290 °C.

Petrological studies in the Námafjall-Krafla area show compositions ranging from olivine tholeiites to rhyolites. Within postglacial time all these rock types have been erupted. The basalts of the fissure swarm through Námafjall and Krafla are quartz normative tholeiites which seem to be of almost identical composition throughout the region.

Axarfjörður is a down-faulted trough 25 km broad that has subsided at least 1,000 m relative to its uplifted western margin at Tjörnes during the Brunhes magnetic epoch². Volcanism is unknown within the Axarfjörður depression but has occurred along its western and eastern margins. The fault swarm which passes through Námafjall and Krafla runs into the eastern part of the Axarfjörður depression.

Historic volcanic activity

Historical accounts mention only one eruption in the Krafla-Námafjall fissure swarm in 1724-1729. Contemporary descriptions of this eruption, the Myvatn fires, are available¹². On 17 May 1724, a row of explosion craters formed. The eruption was mainly from one main crater, Víti, which ejected both silicic pumice and basaltic scoria,

but mostly mud and rock debris from the near-surface rocks¹³. This eruption lasted only a day or two. In 1725, on 11 January, earthquakes occurred and ground fissures opened along the swarm from near Krafla southwards. Steam and mud craters formed in the Krafla and Námafjall geothermal fields, with little or no volcanic activity. Earthquakes were felt throughout 1725. No further significant activity was reported until August 1727, when a fissure eruption started in Leirhnjúkur near the centre of the caldera. A pulse in this eruption occurred in April 1728, when the eruptive fissure extended southwards to Námafjall. Another pulse occurred in December 1728. The lava continued to flow until September, 1729, when the eruption stopped. The lava flows cover about 35 km² and the total length of the discontinued volcanic fissure is 11 km (Fig. 2).

A minor volcanic eruption was reported on this fissure in 1746, accompanied by earthquakes and ground movements, but the eruption site or products have not been located.

A similar course of events was observed during the 1874-75 activity of the Askja caldera and the associated fault swarms^{12,14}. In February 1874, increased steam emission was observed in the Askja caldera. Earthquakes and fissure movement were first observed during the autumn of 1874, 50 km north of the Askja caldera itself¹⁴. In January 1875, a small eruption occurred in the caldera, followed in February by a basaltic eruption within the fissure swarm, 50 km north of Askja, which continued intermittently for 8 months, accompanied by intense earthquake activity and considerable fault movement. Within the Askja caldera a major silicic tephra eruption on 29 March produced approximately 0.5-0.7 km³ of ash, calculated as solid rock¹⁵, and a caldera of about 2 km³ volume collapsed within the pre-existing caldera¹⁵.

In 1618 earthquakes and faulting, lasting several months, in the fault swarm west of the Krafla-Axarfjörður swarm were recorded (Fig. 1) (ref. 16).

The initial seismic activity

Conspicuous seismic activity was noted in the Krafla caldera during early 1975. Towards the autumn, when earthquakes approaching magnitude 4 occurred there, it became apparent that this activity was unusually high. The number of seismograph stations was therefore increased, and in October 1975 seven stations were in operation within 60 km of Krafla. Furthermore, a local civil defense committee was set up and an emergency plan was designed in case of a volcanic eruption.

In the morning of 20 December 1975, the seismic activity increased markedly. Shortly after 10 a.m. continuous tremor was seen on the seismograph closest to Krafla, accompanied by an intense earthquake swarm that soon saturated the seismographs. When this increase in activity was noticed an eruption warning was issued to the Civil Defense Authorities shortly before 11 a.m. At 11:08 an eruption started near Leirhnjúkur in the centre of the Krafla caldera^{17,18}.

The vigour of the eruption decreased quickly and at the same time the seismic activity, that started near Leirhnjúkur, propagated northward along the fault swarm. Within 2 h at least 40 km of the fault swarm had been activated.

The earthquake swarm that followed the eruption continued for several weeks but after the first few hours the activity was mainly confined to two separate areas: inside the Krafla caldera and in the Axarfjörður region (Fig. 1). In both areas the magnitude of the largest earthquakes continued to increase until mid-January. In the Krafla caldera the largest earthquakes occurred on January 6 and 19 (magnitude 5.0), but in the Axarfjörður region the largest earthquake (magnitude 6.3) occurred on January 13, 1976, and caused considerable damage in the village

Kópasker and vicinity. From 20 December to mid-February, during the most intense earthquake swarm, 36 earthquakes of magnitude 4.5 or larger and six earthquakes of magnitude 5.0 and larger occurred.

The epicentres in the Krafla area during this time were distributed throughout most of the caldera. Most of these earthquakes seem to be related to some process within the caldera itself, rather than the fault swarm that crosses the caldera from south to north. Most of the hypocentres were between 0 and 4 km deep, and a few were as deep as 10 km.

In the Axarfjörður area, however, there was a clear relationship between the distribution of epicentres and known fault systems. Epicentres were distributed along the north-south trending fault swarm from 66°00'N to 66°14'N, where the epicentre belt took a north-westerly trend to follow the Grímsey fault of the Tjörnes Fracture Zone (Fig. 1). The large earthquake on 13 January took place near the junction between these two fault systems.

The eruption and the lava

At about 11:25 a.m., 20 December 1975, an eruption was reported in Leirhnjúkur from an overflying aeroplane. At 10:25 pilots of the same plane had seen no sign of an eruption in the area. Other observations from aeroplanes crossing the area revealed that the eruption probably started at about 11:08 a.m.^{17,18}. The first reports and photographs taken at 11:25 a.m. show that most of the lava had already been erupted.

The eruptive fissure is about 2 km long but lava was erupted from the northern half only, where three small individual lavas were erupted with a total surface area of 0.36 km². The new craters formed on the same fissure as was active during the eighteenth century eruption.

The first visitors to the eruption site at noon saw only relatively minor movements at the lava margin. However, they witnessed fault movements and the opening of a mud crater on a fault which cuts through Leirhnjúkur. In the evening of 20 December, very minor volcanic explosions were observed in the craters; the explosions ceased altogether during the following night. After the lava emission stopped the craters continued to emit water and steam, at first, with considerable force, then rapidly decreasing. Only minor steam vents are still active along the fissure.

The lava is an extremely vesicular pahoehoe type lava, with average thickness of <1 m. No cones were formed and there was no ash production. This lava is homogenous in composition and identical to other basalt lavas that have been erupted from this fissure swarm in postglacial time. It is basalt (quartz normative tholeiite) with occasional glomerophytic plagioclase and rare microenphenocrysts of olivine.

Horizontal and vertical movement

Movement along pre-existing faults was noticed very soon after the earthquake activity began. Some idea of the horizontal and vertical movement could be gained by measuring newly formed cracks. The width of individual cracks was measured and the aggregate movement deduced from measurements on profiles across the fault swarm (Fig. 1). In the Krafla area, rifting and vertical fault movement were both about 70 cm. In the Axarfjörður area, the aggregate widening of the fault swarm was 1.0–1.5 m on 30–40 individual faults¹⁷. Subsidence within the fault swarm in Axarfjörður is estimated to be at least 1 m.

Field inspection of the fault swarm between Axarfjörður and Krafla at 5-km intervals showed that the major movements were confined to the Axarfjörður district and the Krafla caldera, with minor displacements between these areas. South of the Krafla caldera, fault displacements of 5–10 cm occurred between Námafjall and Lake Myvatn.

The fault direction is 20°E. Most of the faults indicate a simple opening movement, but occasional faults have

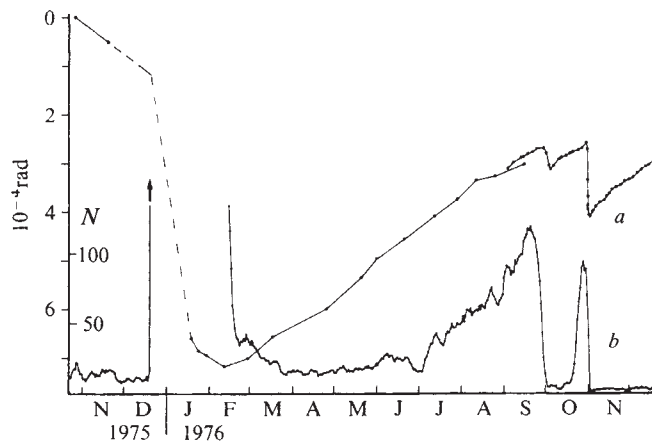


Fig. 3 Correlation between (a) the tilt of the Krafla power house and (b) the seismic activity within the caldera. The north-south component of tilt is shown, (increasing numbers mean tilt down towards north). The tilt corresponds well with the land elevation. *N* is the 5-d running average of the number of earthquakes per 24 h recorded at the seismic station near Reykjavík (Fig. 2) with amplitude above a certain threshold. From 20 December 1975, to 14 February 1976, *N* was >130 and most of that time >1000. The tilt measurements before 20 August were done by optical levelling by engineers of Thoroddsen and Partners, engineering firm.

vertical displacements of up to 60 cm. Movements connected with the large Kópasker earthquake of 13 January affected faults east of Axarfjörður, although the epicentre of the earthquake was off the coast.

Levelling carried out in the southern part of the Krafla area in 1974, and in early March 1976, indicated that considerable subsidence had occurred. The maximum measured subsidence of 2.1 m relative to the north end of Lake Myvatn was found near the eruption site. Further, it was noted that a 70-m long building at the power station inside the southern part of the caldera (Figs. 2 and 3) had tilted about 700 μ rad to the north relative to measurements made 2 months before the eruption.

Later events in the Krafla area

After the culmination in January 1976, seismic activity gradually decreased in both the Axarfjörður and the Krafla areas. In March 1976, the events in the Krafla area took an unexpected course and since then there has been a significant interplay between land elevation changes, fault movements, earthquakes and bursts of volcanic tremors within the Krafla caldera and the associated fault swarm.

Soon after the large earthquake swarm was over, the area started rising again (Fig. 3). The rate of uplift was relatively constant, 6–7 mm d⁻¹, at its maximum near the centre of the caldera, decreasing gradually outwards to <1 mm d⁻¹ 10 km south of the centre. In May, a slow increase in the frequency of earthquakes was noticeable. Measurements showed that the land had regained 50% of the original subsidence by the end of September, and the earthquake frequency had increased by a factor of 10 as compared with the minimum in April. Then a sudden drop in the seismic activity was noticed and at the same time subsidence occurred at a relatively high rate, lasting 6 days and amounting to about 15 cm near Leirhnjúkur. During the period of highest subsidence rate, bursts of continuous tremor were seen on the seismographs and small earthquakes were recorded, originating in the fault swarm about 15 km north of the caldera, on the Gjástykkí faults. On 4 October, the subsidence stopped, and the area started rising again at a similar rate as before.

The seismic activity remained very low until the land elevation approached the same level as before the last subsidence. Then the earthquake frequency increased sharply to almost the same level as before. The elevation had

slightly passed the level of 30 October, when further subsidence started, lasting about 48 h, and the area around Leirhnjúkur subsided about 50 cm. The subsidence rate was much higher than in the previous event and was accompanied by continuous tremor on the seismographs. Simultaneously, fissures in the middle of the caldera showed significant contraction. About 5 h after the beginning of the subsidence a large earthquake swarm (not shown in Fig. 3) started again in Gjástykkí north of the caldera. People travelling in Gjástykkí felt earthquakes and continuous tremor and saw a cloud of steam emanating from a fissure. Later inspection revealed fresh fault movements and a new thermal area in Gjástykkí. The earthquake swarm lasted 2 d, and as soon as the earthquakes stopped in the Gjástykkí faults the land started rising in the Krafla caldera, this time at a similar rate to before. The earthquake frequency within the caldera is, in late December 1976, at a minimum, but is expected to increase sharply when the elevation approaches the level of 30 October.

Boreholes have recently been drilled down to 2 km in the Krafla area. One of these had been blowing for 4 months before the eruption. In April 1976, the CO_2 content of the aquifer water had increased from 200 to 20,000 p.p.m. The CO_2 content of the borehole has since lowered somewhat but is still 10–20 times higher than before the eruption. At the same time, the pH dropped from 8.6 to 1.9 in the discharge from another uncontrolled borehole, and has since returned to its previous value.

The composition of gas emitted from fumaroles has changed significantly towards increase in CO_2 , relative to H_2S and H_2 . The appearance of new fumaroles and increased activity of others indicates a significant increase in the thermal output of the area during the last few months.

Boreholes at Námafjall extend down to 1.8 km, and a considerable amount of data are available on these holes. Measurements since the events of 20 December 1975, have so far revealed no change in composition compared with earlier measurements.

Discussion

The main significance of the activity taking place now in northeastern Iceland is the light it throws on the mechanism of rifting and the associated magmatism. It demonstrates that the rifting long predicted for Iceland as a part of the oceanic rift system is episodic rather than continuous. The historical record shows that the interval between these episodes is of the order of 100–150 yr. Each rifting episode has a duration of several months to a few years, and extends over 50–100-km distance along the rift zone. The present episode started near the south end of the zone that was activated but propagated rapidly along the fault swarm. Seismic activity and faulting have since been primarily concentrated near the ends of the active rift; the central volcano Krafla in the south and the intersection of the North Iceland Rift Zone and the Tjörnes Fracture Zone in the north. Seismic energy release has been greater near the northern end of the zone than near its southern end, but the northern end now seems to be coming to a rest while the southern end is still active 12 months after the rifting started.

This rifting, like that in the eighteenth and nineteenth centuries, is closely connected with magmatism. The causal relationship between rifting and magmatism is, however, unclear.

The eruption of 20 December 1975, is only a minor manifestation of the total magma movement. The volume of the lava erupted ($3 \times 10^5 \text{ m}^3$) is much smaller than the volume of the initial subsidence bowl, which can be estimated at 10^8 m^3 . The subsidence is interpreted as a result of the widening of the rift system and a movement of magma from the Krafla area along the fault swarm to the

north, presumably along a horizontally fed dyke opened by the rifting process.

Later, in March 1976, the Krafla area started inflating, but the inflation has twice been interrupted by short subsidence events. At the end of September and the end of October (Fig. 3). These subsidence events are of a similar nature and it is probable that the difference between these and the large event that started on 20 December 1975, is only in degree.

The inflation is interpreted as being caused by magma transported from below accumulating at a shallow depth within the Krafla caldera. Simple model calculations indicate that the inflow of magma into the roots of the caldera since March is approximately $5 \text{ m}^3 \text{ s}^{-1}$.

The deflation is caused by release of pressure in the magma chamber by widening of the rifts and flow of magma into the fault swarm north of the caldera. This flow is accompanied by volcanic tremors, earthquakes, faulting and thermal activity in the fault swarm. In this model the earthquakes in the caldera are caused by inelastic deformation of the crust above the magma chamber.

Further support of this model comes from work in progress on the distribution of earthquake hypocentres within the caldera and the attenuation of S waves, which strongly suggests the presence of a magma chamber below the depth of 3 km. The low pH value measured in the borehole fluid last April and the increase in CO_2 are interpreted as being a result of the influx of magmatic gases into the hydrothermal system.

Judged from previous historical events, years may pass before the area comes to rest. Therefore, and because of the economic importance, great emphasis is put on continued monitoring of the area, including telemetering of signals from three seismometers to an observatory where they are watched around the clock. The tilting of the Krafla power house is recorded twice a day and the ground movement is measured by optical levelling and gravimetry every 2–4 weeks. The movement of faults is monitored. The composition of borehole discharge is monitored regularly. The gases emitted from fumaroles are analysed every few weeks. Ground temperatures are measured and a close watch is kept on the thermal fields. Plans have been tested so the area can be evacuated at a very short notice in case of abnormal seismic activity.

From the data accumulated so far a better understanding of the rifting mechanism and the associated magmatism is rapidly emerging. It is obvious already that the data will reveal many of the detailed features associated with the formation of the Icelandic crust.

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Note added in proof: On 20 January 1977, the floor of the Krafla caldera subsided again about 30 cm in 20 h. This was accompanied by seismic activity, fault movements and development of a new steam field within the fault swarm, Gjástykkí. The centre of the caldera started to inflate immediately after this subsidence and another subsidence period is expected in early March 1977.

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Saturated platelets are new intermediates in hydrocarbon pyrolysis and carbon formation

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Formation of solid carbon during pyrolysis of gaseous hydrocarbons may involve 'saturated platelet' intermediates.

AN important problem in flames and in the industrial pyrolysis of hydrocarbons is the understanding of how solid carbon is formed, either in the gas phase (soot) or on surrounding surfaces. Various mechanisms have been discussed^{1,2}, but none takes full account of soot ball internal structure, electrical phenomena and the startling temperature range (1300–2300 K) of soot formation. This article proposes a single route to soot for all conditions, passing through sequential chemical and physical steps.

Small intermediate molecules

To explain soot formation from varied starting hydrocarbons, possible common intermediate molecules proposed are carbon vapour (C, C₂, C₃, and so on), benzene (and other aromatics), and acetylene^{3,4}. Gaydon and Wolthard¹ have convincingly criticised the carbon vapour route at lower temperatures. Certainly the band radiation of C₂ is not evident in pyrolysing hydrocarbons at lower soot-forming temperatures⁵. It has been suggested that benzene and other aromatics condense chemically to form the polyaromatic molecules found in flames⁶, and hence soot. During pyrolysis of benzene, however, benzene absorption disappears well before soot-like luminosity is seen, without the appearance of polyaromatic bands⁵. Concentrations of polyaromatics in a premixed flame continue to rise slowly through the sooting region, indicating that polyaromatics are byproducts rather than intermediates of the sooting process⁷.

Porter^{3,4} states that acetylene is "the last stable hydrocarbon to be observed before carbon formation". It is formed in appreciable quantities in most pyrolyses above 1300 K, and is still relatively stable at the highest soot-forming temperatures (2300 K). Temperature measurements of the pyrolysis of methane show that an overall endothermic process is followed by an overall exothermic process⁸. This is consistent with strongly endothermic reactions to acetylene, followed by exothermic steps to soot. Porter has outlined processes which would begin with acetylene and end with soot. Obviously some polymerisation will occur (C₂→C₁₀) and some dehydrogenation (H/C from 1 to 0.1 atom ratio), but the sequence of these steps will be governed by the chemical kinetics. The high transition-state entropy decrease found with typical polymerisations, and the higher activation energies found with decompositions suggest that most decomposition reactions will be faster than polymerisations for temperatures higher than 1300 K (ref. 3). Porter concluded that little polymerisation could occur at sooting temperatures without some dehydrogenation, and that they were simultaneous processes and intimately connected.

Saturated platelets

If acetylene is the common small intermediate in soot formation, it is probable that there are larger intermediates formed before soot, for a study of soot formation in heated acetylene showed

that the soot balls continued to grow in size even after all the acetylene had been removed⁹. Optical absorption evidence in an oxygen-acetylene flame showed that all the soot substance was present in some larger (absorbing) intermediate before much soot was detected¹⁰, and cooled-probe tar collection in benzene pyrolyses¹¹ gave similar indications.

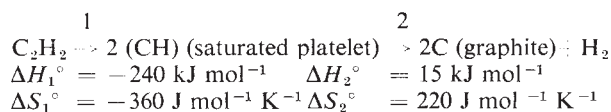
A continuum absorption has been seen in pyrolyses and diffusion flames of hydrocarbons⁵. This absorption was probably due to larger soot precursors or intermediates because it was seen in all pyrolyses and flames before sooting occurred, and it was not seen in the soot-free pyrolysis of methyl alcohol. These precursors of soot are probably generally formed from acetylene, for the continuum was found at lower temperatures in the pyrolysis of acetylene than with other hydrocarbons (including benzene). In a mass spectrometry study of transient species in low-pressure acetylene-oxygen flames⁷, two types of species peaked in concentration before soot was found—polyacetylenes (C_{2n}H₂), and a group of much larger molecules in the mass range 300–500. These latter were found in very low concentrations (10⁻⁷ mole fraction) compared with the polyacetylenes, suggesting a route through polyacetylenes to soot, although the larger molecules could also contribute. Polymerisation of long rod-like polyacetylenes at higher temperatures seems unlikely, however, as configurational entropy and activation energy differences probably again favour decomposition reactions. Mass spectral data suggest that the larger molecules lost hydrogen continuously before or during sampling. By collecting 'young soot' on cooled tubes in the flame, the hydrogen-carbon ratio of the substance was found to be as high as 1.

These observations can be explained if one postulates that structures of the type shown in Fig. 1 are formed directly from acetylene. They retain the H-C ratio of acetylene at lower temperatures, and have a puckered layer of carbon atoms bonded by single bonds, except at the edges. Hence 'saturated platelets' is an appropriate term for them. They can retain radical sites, and acetylene can add on by a radical mechanism. Hydrogen atoms are held above and below the carbon layer on alternate carbon atoms, and can be lost as H₂ from the interior of the platelet by a radical mechanism. Hence graphite-like layers are formed from the inside, moving to the outside, and simultaneous addition of acetylene and dehydrogenation is possible in the same molecule at different sites in excellent agreement with Porter's prediction. If the dehydrogenation is more rapid than addition, only a perimeter of hydrogen atoms remains and one has the polycyclic aromatics observed in flames. Acetylene pyrolysis in conditions where no breakdown of the carbon skeleton of platelets is expected¹² (small hot wire immersed in cold acetylene) yielded an oily deposit on the walls, consisting of "a series of heavy polynuclear aromatics containing even numbers of carbon atoms". This is good evidence for the direct build-up of acetylene into these larger species.

Evidence supporting the existence of saturated platelets is the observation¹³ of arrays of 0.154-nm (singly-bonded) C-C bonds by electron diffraction of 'young soot', in addition to the

0.141-nm graphite-like C-C spacings. Also, 'young' soot has 100 times the electron spin resonance strength of 'old' soot sampled further downstream in the flame⁷, indicating many radical sites on the young soot. This is consistent with the presence of saturated bond areas on the adhering material which neutralise free radicals in the process of dehydrogenation to the more stable aromatic form.

The thermodynamics of platelets, calculated from group properties, can be summarised thus (see also note added in proof)



all at 300 K. ΔG_1° is negative for temperatures < 650 K, whereas ΔG_2° is negative for all temperatures > 70 K. Saturated platelets can then be expected to be short-lived at flame temperatures before dehydrogenation occurs. The kinetics of acetylene disappearance and the appearance of ethylene, benzene and so on,

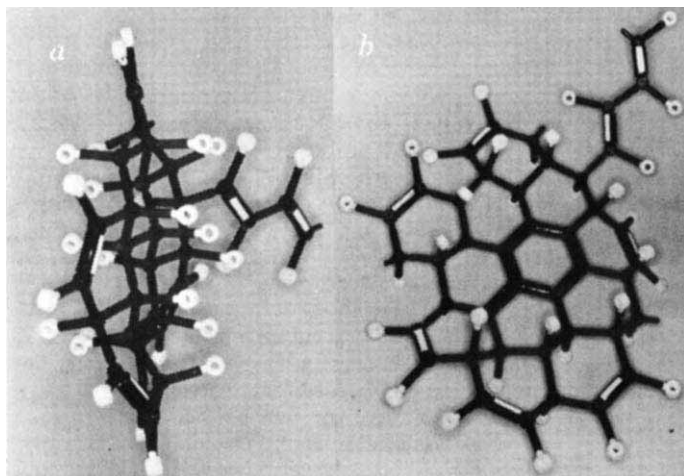


Fig. 1 *a*, Model of a $C_{27}H_{27}$ saturated platelet, with attached C_4H_5 radical chain, showing the puckered carbon skeleton and distorted general shape. The $C_{27}H_{27}$ heat of combustion $Q = 330 \text{ kJ mol}^{-1}$ C atoms, equal to that of a bituminous coal¹¹. *b*, Model of a $C_{38}H_{38}$ platelet with attached C_4H_5 radical chain, showing a central aromatic region formed by dehydrogenation. The $C_{38}H_{38}$ $Q = 365 \text{ kJ mol}^{-1}$ C atoms, higher than that of a bituminous coal^{11,15} but accounting for several C-O bonds restores Q to that of coal.

at the lower temperatures has been interpreted in detail in terms of radical platelets. This analysis will be published elsewhere, but it is sufficient to note here that the 'polymer' of composition $C_{n2}H_{n2}$ found by Silcocks¹⁴ in particular corresponds well with the existence of saturated platelets.

Control of platelet formation and growth

Formation of saturated platelets probably begins with the formation of benzene (or a derivative) and then naphthalene (or a derivative). Experiments with nitric oxide¹⁴ indicate a radical mechanism for acetylene disappearance, so a phenyl radical and then larger radicals are likely intermediates on the route to a saturated platelet radical (Fig. 2). The disappearance of acetylene can, in fact, be explained only by the involvement of a large molecule. The kinetics of disappearance are second order over the extraordinary temperature range 650–2500 K (ref. 15). Bimolecular mechanisms which are currently favoured¹⁵ utilise small radicals such as C_2H , especially at high temperatures. Reactions with five to seven atoms in the transition state, however, become energy-transfer limited at temperatures in the vicinity of 1000 K (ref. 16). Hence a small radical-acetylene reaction which was second order at lower temperatures

should become third order at temperatures not much higher than 1000 K. If the radical is attached to a large enough body (platelet), the decomposition should remain second order to very high temperatures, as observed.

Soot formation may be limited at the higher temperatures by the initial steps of producing phenyl (C_6H_5) radicals or similar aromatic radicals, as expected from entering an energy-transfer-limited regime. This would explain why, in premixed flames, naphthalene and benzene derivatives produce soot at lower fuel-air ratios than acetylene¹. This 'sooting tendency' in competition with oxidation has been used to argue for the aromatic polymerisation route, and against the acetylene mechanism, but the survival of some aromatic radicals derived from benzene or naphthalene fed to the flame may bypass the otherwise limiting step of making benzene or naphthalene and allow a faster build-up of acetylene on these radicals to form saturated platelets.

In shock tube studies¹⁷ of benzene, toluene, ethylbenzene and indene in the temperature range 1600–2300 K, soot particles were identified by the scattering of light of 488 nm wavelength, but there was also absorption of this light in the pyrolysing mixture before scattering occurred. There was absorption at 632.8 nm which increased later than that at 488 nm, entirely consistent with the absorption found by Parker and Wolfhard⁵—a continuum spreading from the ultraviolet into the visible spectrum. Measurements of the ratio of absorption at these two wavelengths¹⁷ show that the ratio reached a steady value (when dehydrogenation had ceased) close to the time soot particles appeared. Again, these observations are consistent with the formation of saturated platelets which are dehydrogenated to the point where they are agglomerated to soot particles. The continuum spreading into the visible is likely to arise from increasingly conjugated double bonds, either in chains attached to the platelets or integral with its structure, on an edge.

The finding that this absorption reached a plateau which was lower (lower soot yields) with higher temperature in contrast with the higher rate of absorption increase with higher temperature, was interpreted¹⁷ as indicating competition between a rapid direct soot formation from aromatics, and a slower indirect route to soot by fragmentation reactions (for example, through acetylene). An alternative explanation is that the absorption measured platelet concentration rather than soot during its initial increase, and that initiation of platelets, which occurred almost solely at the beginning of the shock period when hydrocarbon concentrations were high, has a negative temperature coefficient because of a rapid route through an aromatic (unstable) vying with a slower acetylene (stable) initiation. Thus, as the temperature was raised, fewer platelet initiations occurred, resulting in a lower conversion to absorbing platelets and soot. Growth of each platelet was more rapid with rising temperature, however, showing a more rapid rise of absorption.

Agglomeration of platelets

A mechanism proposed for agglomeration of larger intermediates is physical nucleation and condensation into liquid droplets. This was supported by Lahaye, Prado and Donnet¹⁸. If liquid condensation occurs at all, however, it must be limited to the very lowest of sooting temperatures. The large molecules found by Homann and Wagner⁷ correspond to C_{25} – C_{40} polycyclics. The condensation points of these molecules can be estimated—the polycyclic perhydrochrysene ($C_{18}H_{30}$) has a boiling point (1 atm) of 630 K (ref. 19) and the condensation point of the C_{40} molecules is likely to be no greater than 1100 K (by extrapolation of Fig. 35 from ref. 20). Johnson and Anderson⁹ collected 'polymer drops' from the pyrolysis of acetylene, but these only appeared when they sampled in regions with the temperature below about 570 K. They concluded that any drops observed after pyrolysis had been condensed on to small carbon particles and then only when the pyrolysis gas was cooled below 570 K. It is thus unlikely that a liquid droplet route can operate in the formation of soot balls.

A number of observations support an electrical mechanism of soot ball nucleation. An increase in soot particles deposited on electrodes from a diffusion flame was found after seeding with cesium chloride²¹. When soot formation was promoted and positive hydrocarbon ion concentrations were elevated by adding carbon tetrachloride in a diffusion flame²², there was a local depletion of ions where soot was forming. The concentrations of large positive hydrocarbon ions in premixed flames have been shown to peak just upstream of soot formation²³, and many of the younger soot particles were positively charged²⁴.

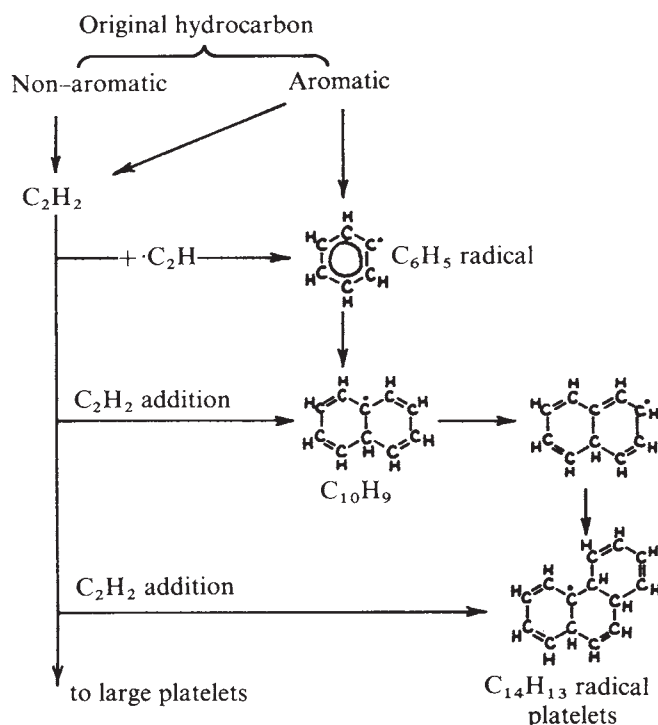


Fig. 2 Initial steps in the formation of a saturated platelet by radical addition of acetylene.

The necessity of electrical charges playing some part in soot formation has been challenged^{1,25} with the observation that in the absence of a flame and its non-equilibrium ions produced by chemi-ionisation, ionisation should be negligible, and a pyrolysing hydrocarbon is not expected to soot by an electrical mechanism. There is electric probe evidence of charge carriers being generated in pyrolysing hydrocarbons in the absence of a flame in heated propane and methane²⁶. Above 1330 K the probe current increased rapidly and carbon deposited only on the anode; during ethylene pyrolysis²⁷ at the lower temperature of 1100 K, with much higher electric fields, carbon deposited only on the cathode. Current transients from a probe immersed in shock heated ethylene indicated an ion recombination after the initial ionisation. These studies indicate that a flame is not necessary for the appearance of charge carriers related to soot formation, and suggest that the ions formed by the oxidation process itself are not necessary for some, at least, of the soot formation in flames.

At higher temperatures it seems that negative charge carriers were carrying more carbon than positive charge carriers²⁸, suggesting the existence of large negative hydrocarbon ions. Ion-pair processes $AB \rightarrow A^+ + B^-$, or ionisation $A \rightarrow A^+ + e$ followed by a rapid capture of the electron may well occur in hydrocarbons at these relatively low temperatures. Possible steps involve $C_3H_3^+$ ($I = 5.8$ eV, refs 28, 29) and C_2H^- (E.A. = 2.2 eV, see ref. 30), both of which have been found in flames^{31,32}. Dehydrogenating platelets, however, have the

possibility of creating aromatic edge sites with σ -electron affinities similar to those of C_6H_5 , $C_{10}H_7$, $C_{14}H_9$ radicals (2.2 eV or 1.2–1.6 eV (refs 33 and 34)). Thus negative platelet ions are good candidates for the large ions mentioned above.

Evidence for generation of large negative hydrocarbon ions in pyrolysing hydrocarbons is indirect because studies have been done only in flames, for which an alternative interpretation is available—that electrons diffuse to the pyrolysis zone from the combustion zone, and attach to form negative ions. Large (up to the mass spectrometer limit, 130 mass number) negative ions have been found³⁵ on the fuel side of a diffusion flame. The validity of the electron diffusion interpretation is being checked by a similar study on pyrolysing hydrocarbons.

Thus, let us assume that a weak plasma exists in a pyrolysing hydrocarbon, consisting of small positive ions, large negative platelet ions and neutrals. Some of the neutrals will be platelets, and a certain fraction of all platelets and smaller molecules will be radicals. It is clear that this combination can lead to electrical-radical nucleation.

(1) Some radicals will attach ions, and on average will attach positive ions more frequently than negative ions (the positive ions are smaller and more frequently bombard other species because of their high velocities).

(2) The predominantly positive radical-ions attract and hold a negative platelet by an electrostatic force, resulting in a directional approach and instantaneous bonding.

(3) The resulting neutral radical will on average acquire another positive charge by process (1) (bombardment charging).

(4) A further electrostatic neutralisation as in (2) will occur.

This cycle will be repeated until sufficient radical sites will have been acquired so that neutral platelets begin to be attached (chemically) in appreciable numbers. The nucleation has then ended, and normal growth of the soot ball has begun, with the charge still predominantly positive, but having little influence on the growth. A factor which could also be important in maintaining the positive charge of nuclei relative to smaller gaseous positive ions is the polarisation energy of the packed platelets (that of aromatic crystals is about 1.5 eV (ref. 36)), which will reduce the energy of any ion-pair process originating from the nuclei.

Nuclei growth rate in the above ion-recombination process was calculated for the conditions of Prado's pyrolysis of benzene¹¹ at 1380 K; this gives a platelet collection rate of only 10^{-4} of the average rate required to build up the final 35-nm particles found by Prado. It seems that the growth accelerates after the nucleus is formed, and that the nucleus is very small, for the final particles contain material equivalent to 10^4 platelets. Notwithstanding the large uncertainties in ion concentrations, these conclusions should still hold. It is difficult to determine whether the growth is wholly irreversible. We have evidence from rapid quenching experiments (unpublished) that a gas phase equilibrium is obtained during soot formation which ignores the presence of solid carbon. This would support an irreversible growth, much slower than the prevailing gas kinetics. On the other hand, there must be competition between nuclei for platelets, and in the very early stages where the number of successful nuclei is determined and the process is unlikely to be irreversible, the electrical sphere of influence of each ion may be important. Thus the Debye shielding radius, or the much smaller ion-recombination radius, may determine the final number of soot balls. For Prado's condition¹¹, Debye radius calculations gave $2 \times 10^{16} \text{ m}^{-3}$, compared with an experimental 10^{15} m^{-3} .

The physical form of soot is consistent with the platelet agglomeration ideas presented above. High resolution phase-contrast electronmicroscopy³⁷ has shown that the basal layers in soot particles are aligned parallel to the surface (onion-like), which is easily explained by the wrapping around of platelets deposited from the gas phase. Some layers show interlayer spacing well in excess of graphitic spacing, which is what would be expected from those remaining saturated platelet regions. 'Thermal' carbon blacks—made by the pyrolysis of hydrocarbons in the absence of air—show uniform spherical particles with a

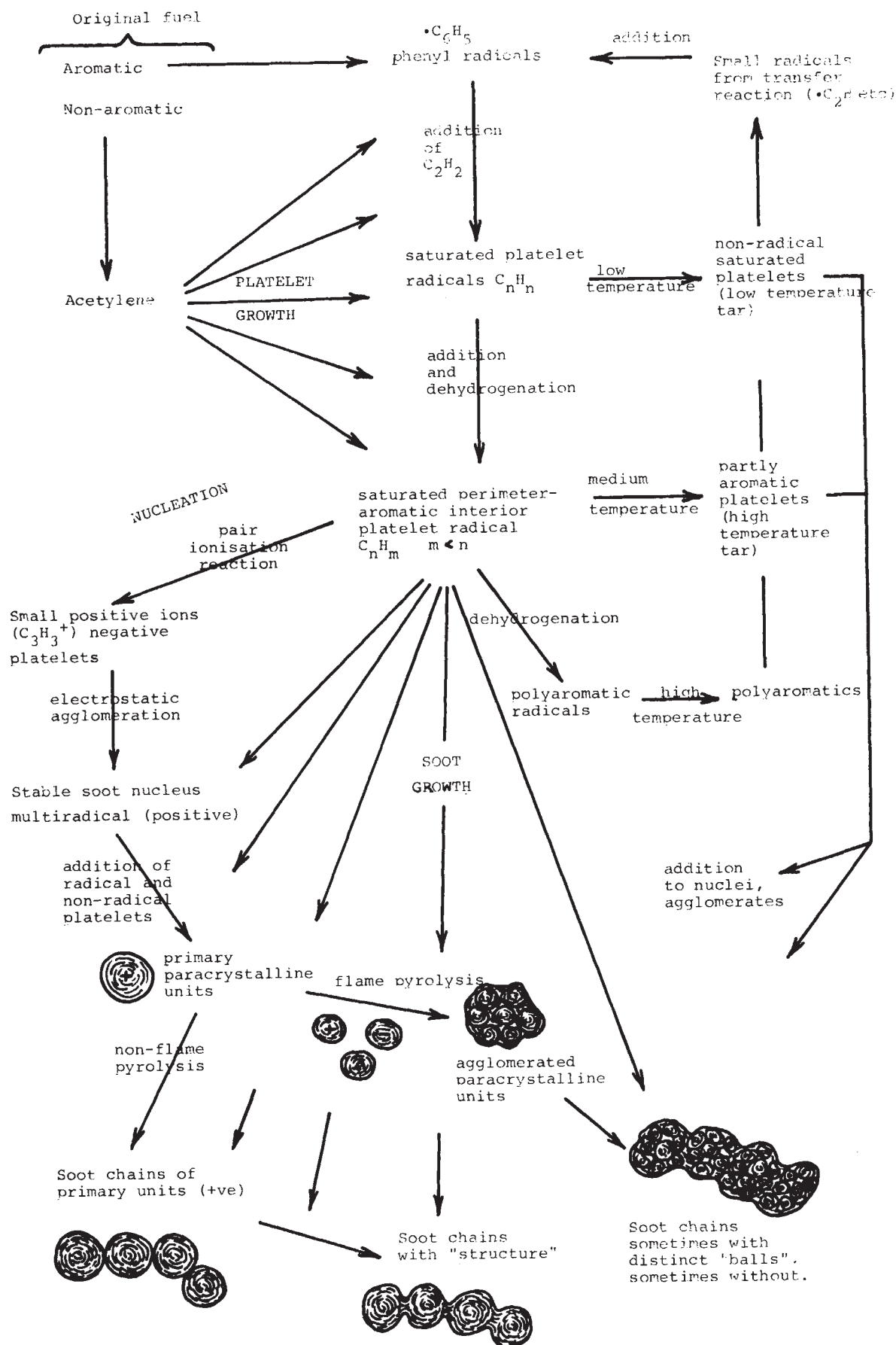


Fig. 3 Steps proposed here in the production of soot from a hydrocarbon fuel. At high temperatures (~ 2000 K) the initial steps are rate controlling, whereas at lower temperatures (~ 1300 K) the electrical stages are controlling. Production of methane and other light hydrocarbons on radical platelets is not shown.

single centre of growth. In contrast, furnace and channel blacks—made in the presence of air—show many more growth centres per unit mass, and these are firmly embedded together by more onion-like layers³⁷ around the outside. Wersborg *et al.*^{23,24} observed this latter type in flames, and from the peak in particle numbers with distance it seemed that each growth centre had an independent existence in the gas phase before coming together. The different physical forms may be related to electrically aided or hindered agglomeration in that non-flame pyrolysis probably results in many soot nuclei maintaining a positive charge, hindering agglomeration, whereas in flames the soot nuclei may be positive, neutral or negative²⁴, encouraging earlier agglomeration.

The single-centred or multicentred units are finally found in many soots more or less tightly joined together in long chains. Howard³⁸ described well this final step of agglomeration, on the basis of the directional agglomeration of repelling like charges. All the steps proposed for soot formation are presented in Fig. 3.

Deposition of carbon on walls at high temperatures

The deposition of platelets on walls is likely to be important wherever the hydrocarbon gas phase has been heated sufficiently to generate acetylene. Examples are the conditioning and coking of thermal cracker walls³⁹, the deposition of laminated pyrolytic graphite on hot surfaces, and the extensive rearrangements of structure occurring within hydrogen bearing carbon materials on heating. In the heating of cokes, for example, acetylene formed from residual hydrogen can redeposit from the pore spaces as platelets, which will then constitute a graphitisable portion of the solid.

Many hydrocarbon pyrolysis experiments have revealed the importance of aged walls (platelet-covered walls) for reproducibility of rates. The generation of wall radical sites, radical transfer to gaseous species, and eventual termination perhaps back on the wall as radical platelets, can explain such behaviour, and the occurrence of induction periods. The importance of wall-derived radicals is relatively easily recognised, but electrical effects must also be considered. Abrahamson and Kennedy²⁶ found some electrical behaviour with their metal probe which was interpreted as being surface ionisation, at lower temperatures than that at which appreciable gas phase ionisation occurred. This wall behaviour is consistent with the crystal polarisation effect, whereby the energy of an ion-pair reaction involving a platelet is smaller if that platelet has its electron levels banded with other nearby platelets, as on a wall deposit. The electrical control of wall carbon deposition found by Abrahamson and Kennedy at slightly higher temperatures probably relates only to the initial stages of deposition, but it could well be useful in the commercial control of wall coking.

Deposition of carbon on walls at low temperatures

If wall surfaces can supply free radical sites, and chemisorb products of surface rearrangements, it is possible that saturated platelets may form on the wall surface itself, from small molecules other than acetylene. This route should be observable, if at all, at lower temperatures where acetylene and gas phase platelet formation are unlikely.

Lobo and Trimm⁴⁰ measured deposition rates in the range 650–770 K and found zero order kinetics with a common activation energy for deposition from acetylene, ethylene, propylene and butene. Again, a reproducible rate was obtained only after several⁴¹ carbon layers had been deposited. Plummer⁴² notes that the surface residue formed on nickel and tungsten has the same photoelectron spectra whether deposited from acetylene or ethylene. The residue formed from ethylene above 230 K. On heating to 500 K, hydrogen desorbed from the surface until a $(C_2H_2)_n$ complex remained, which was stable up to 1100 K. The formation of acetylene and hydrogen chemisorbed on to the surface—one interpretation of these experiments—is

not likely to be energetically as attractive as the formation of saturated platelets $(C_2H_2)_n$ together with locally chemisorbed hydrogen. The electronmicroscope observation⁴³ that pre-graphitic deposits formed on platinum catalyst from various hydrocarbons at 633 K covered only distinct tiny patches of the surface supports platelet formation.

These temperatures are similar to those expected for coal formation, and humus formation in soils. I will argue in a later publication that the saturated platelet is the archetypal molecule for both coals and humic substances, and that light hydrocarbons from rotting vegetable matter rearrange relatively rapidly on finely divided soil constituents to form humus, but slowly over extended periods on other platelets to form coal seams.

I thank the Nuffield Foundation for a Commonwealth Travelling Fellowship, and Professor J. F. Davidson and his staff in the Chemical Engineering Department, Cambridge, for their hospitality.

Note added in proof: A recent crystalline orbital calculation⁴⁶ of hydrogen atoms chemisorbed on to a single graphite layer (one side only) indicates that saturated platelets should be stable to temperatures of 300 or 400 K, rather than 70 K.

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Sequence and structure of D-glyceraldehyde 3-phosphate dehydrogenase from *Bacillus stearothermophilus*

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The glyceraldehyde 3-phosphate dehydrogenase holoenzyme of Bacillus stearothermophilus possesses precise 222 symmetry: in this respect it differs from the reported structure of the lobster muscle enzyme. Pairs of active sites are linked through a flexible polypeptide loop which probably mediates the structural changes giving rise to cooperative effects. Three additional salt bridges made by each subunit to others would make a major contribution to thermostability of the tetramer.

MANY current ideas about the cooperative nature of protein transitions have derived from studies of D-glyceraldehyde 3-phosphate dehydrogenase (GPDH). The structure and mechanism of this enzyme have been investigated for several species (for a review see ref. 1). All known GPDH molecules are tetrameric with a molecular weight of 145,000 and bind four moles of the coenzyme, NAD. Although the four subunits are chemically identical² the affinity of NAD for the muscle enzyme decreases with each additional NAD bound. The first two moles of NAD are bound tightly and the second two loosely³⁻⁵. Yeast enzyme, however, displays a complicated mixture of positive and negative cooperativity^{6,7}.

The model which best describes the binding is controversial. The allosteric model⁸ of cooperativity and the induced fit model⁹⁻¹⁰ differ with regard to the preservation of symmetry at various states of ligation. Both models assume complete symmetry for the non-ligated and fully ligated states, but the induced fit model allows asymmetry for intermediate states. A model for GPDH has been proposed¹¹ in which the molecule is never fully symmetric, but consists of an asymmetric pair of symmetric dimers, and this view has received support from crystallographic studies of the lobster enzyme¹².

The enzyme from mesophiles becomes unstable once NAD is removed. Because of its greater stability, GPDH from the thermophile *Bacillus stearothermophilus* can be crystallised with a range of NAD stoichiometries from zero to four moles per tetramer¹³. The structure and properties of the bacterial enzyme are very similar to those of the muscle enzyme¹⁴⁻¹⁶.

We report here the complete sequence of 333 amino acid residues in the subunit polypeptide together with the interpretation of an electron density map at 2.7-Å resolution of the holoenzyme with four moles of bound NAD. For the first time it is possible to compare the structure and sequence of a multi-subunit protein from both a mesophile and a thermophile. Our structure differs in some important respects from the structure of the lobster muscle enzyme^{12,17,18}, especially with regard to molecular symmetry.

Experimental methods

B. stearothermophilus GPDH was purified for crystallography by a modification of the method of Suzuki and Harris¹⁵, and for

sequence work according to Hocking and Harris¹⁹. The sequence of the enzyme was determined largely (85%) by the automated Edman degradation technique with the aid of both spinning-cup and solid-phase sequencers (see legend to Fig. 1). The preparation, isolation and sequence determination of fragments of the enzyme are described elsewhere²⁰.

Holo-GPDH (with 4 mol NAD per tetramer) was crystallised by vapour diffusion against 2.4–2.7 M (NH₄)₂SO₄. The crystals contain a complete tetramer in each asymmetric unit. Rotation-oscillation data were collected on an Arndt-Wonacott camera²¹. An initial electron density map at high resolution was calculated by isomorphous replacement from two derivatives, and improved by use of the molecular symmetry^{22,23}.

Molecular structure

The complete amino acid sequence (Fig. 1) is highly homologous with that of the lobster enzyme^{1,24}. Of the residues, 51% are identical in the thermophile and lobster enzymes, as compared with 70% identity between lobster and pig, and 68% between the lobster and yeast enzyme¹. The essential cysteine 149 (ref. 25) is retained in a stretch of conserved residues. The number of arginines is increased from nine to 15 and the number of cysteines reduced from five to two. Lysine 183 which takes part in an acyl transfer reaction^{26,27} in muscle apo-GPDH (NAD-free) is replaced by arginine in the thermophile enzyme.

A diagrammatic representation of the whole molecule is shown in Fig. 2. The overall structure of the subunit of the thermophile holo-GPDH is closely similar to that described for lobster GPDH, consisting of the two folding domains shown in Fig. 3. The first domain, residues 0–148, contains most of the residues involved in NAD binding. The secondary and tertiary structural features of the lobster enzyme are reproduced quite accurately. Similar folding domains have been found in other nucleotide binding proteins²⁸⁻³¹. Side chains implicated in catalysis are contained in the second domain, the region comprising residues 149–333. In this domain the interaction of the β -sheet regions, β_5 with β_6 , and β_7 with β_8 , is different from that of the lobster enzyme and gives rise to a modified hydrogen-bonding pattern. This change results in the burying of aspartic acid 293 which has significance for thermostability (see below).

An important feature of this domain is an irregular S-shaped loop of polypeptide chain (residues 178–201) which is in contact with the NAD and with the S loop and the α_c helix of the subunit related by the dyad axis marked R (see Fig. 2). In addition, this S-loop interacts with several amino acids across the P axis.

The sequence of the S loop is highly conserved in the lobster, pig and yeast enzymes, whereas the homology between the thermophile and mesophile S-loop sequences is only average. This is surprising since the S loops form the core of the tetramer, and most of their residues are internal, and essential to the interaction across the R axis. Many hydrogen-bonding groups in the main chain of the S loop seem to be unsatisfied in both the lobster and thermophile enzymes. Conserved residues are similarly oriented in both structures. The conformation of the

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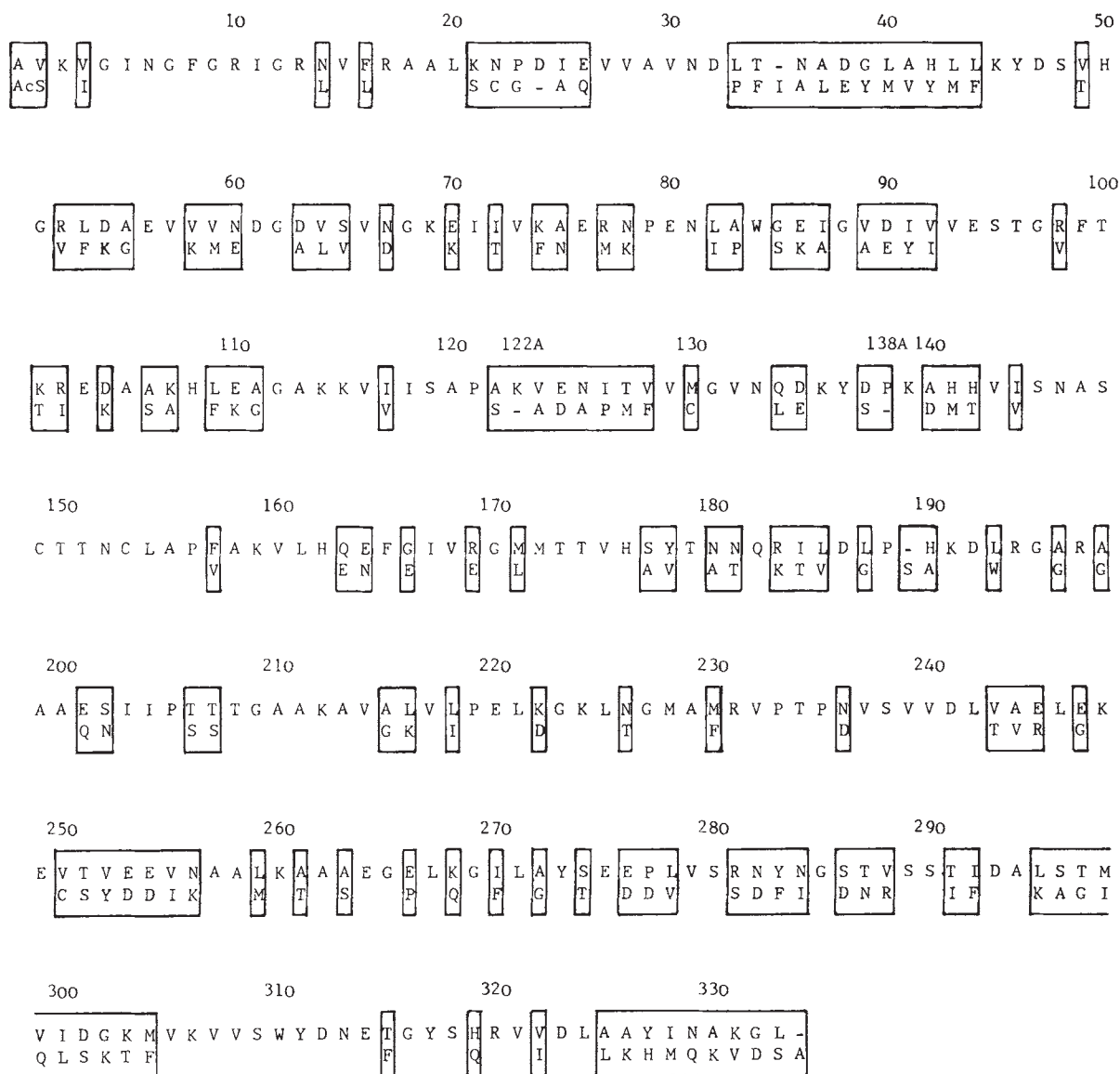


Fig. 1 The primary structure of GPDH from *B. stearrowthermophilus* (upper) and lobster. The sequence of the thermophile enzyme was obtained by automated sequence analysis of fragments obtained from S(2—¹⁴C)-carboxymethylated enzyme with cyanogen bromide, trypsin digestion of citraconylated protein and cleavage of succinylated protein with hydroxylamine⁵³ and with BNPS-skatole⁵⁴. The remainder of the sequence was obtained by the dansyl-Edman degradation⁵⁵ of peptides from sub-digests of cyanogen bromide fragments with trypsin and staphylococcal protease⁵⁶. The sequence of the lobster enzyme is from ref. 24. The residues of the enzymes are numbered to maximise homology between the sequences. Hence the N-terminal residue of the thermophile GPDH is numbered 0; deletions are introduced at residues 35 and 189 and insertions at 122 A and 138 A of the *B. stearrowthermophilus* protein.

S loop appears flexible, but is maintained by the packing of side chains and by interactions with neighbouring subunits made through the contacts across the P and R axes.

Those residues which form the extensive interface between subunits related by the P axis are highly conserved. The Q-axis interface involves few residues but these retain the same specific interactions as in the lobster enzyme³².

Active site

The NAD binding region is shown in Fig. 4. The coenzyme is bound as in lobster GPDH, differences being confined to a few residues interacting with the adenosine portion of the coenzyme. All the residues implicated in substrate binding and catalysis are conserved except asparagine 181. We observe the same two strong electron-dense features which have been interpreted as bound anions and are probably the sites of the phosphate groups of the substrates during the catalytic reaction (Fig. 4).

According to Trentham's mechanism^{33,34} (Fig. 5), glyceraldehyde 3-phosphate reacts with the enzyme to form a hemithioacetal (II), which is converted to an acyl-enzyme (III) with

the transfer of a hydride ion to the nicotinamide C4. After NADH is replaced at the active site by NAD (IV) the acyl enzyme is phosphorylated to give the product 1,3-diphosphoglycerate (V). In agreement with the results of Moras *et al*¹², a model of the hemithioacetal built into the active site with the substrate chain in an extended conformation points the hydride atom of C1 towards C4 of the nicotinamide and puts the 3-phosphate in the 'substrate' anion binding site (P_s in Fig. 4). The Cl oxygen is thought to be protonated by the N⁶ of histidine 76, and the C2 hydroxyl of the D isomer can hydrogen bond to the hydroxyl of serine 148. The L isomer which is not a substrate, cannot bind in this orientation because its C2 hydroxyl would interfere with the imidazole ring of histidine 176. These conclusions agree broadly with those reached for the lobster enzyme.

Three amino acid side chains play an important part in the 'substrate' anion site (P_s). Two of these (threonine 179, asparagine 181) are in the S loop and the third (arginine 231) is surrounded by other side chains of the S loop. The S loop thus connects directly the NAD binding site of one subunit to the catalytic site of the R-axis related subunit.

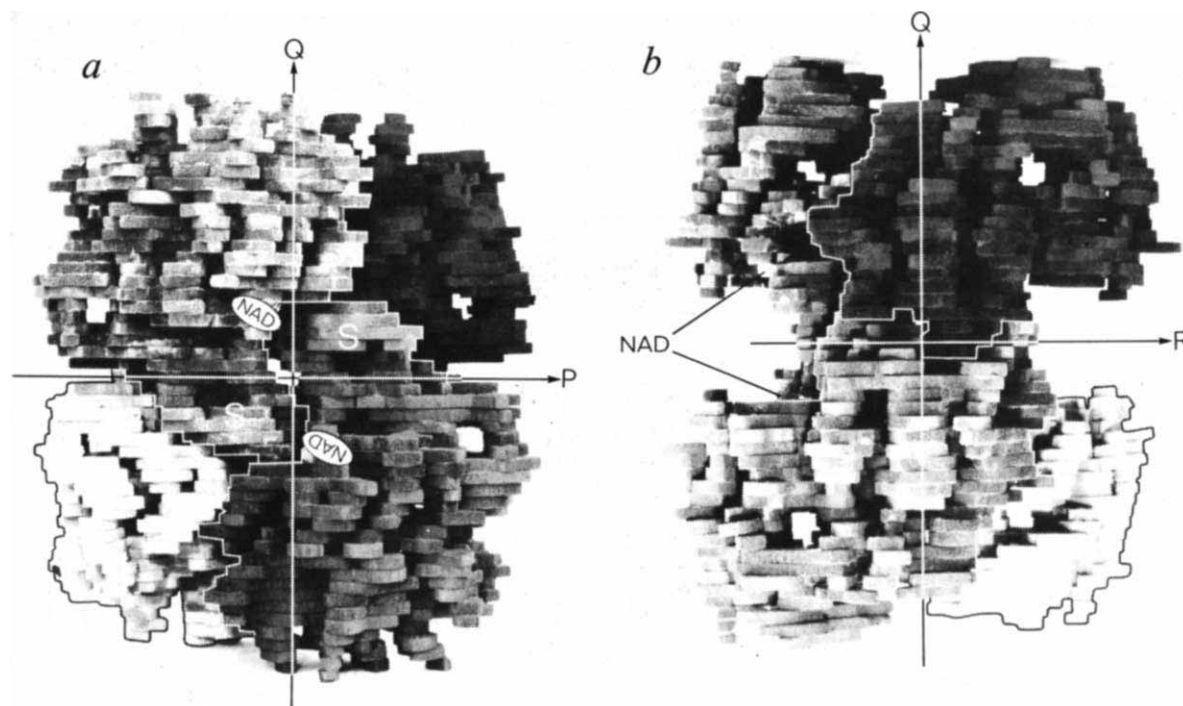


Fig. 2 The four subunits are related by three orthogonal twofold symmetry axes, designated the P, Q and R axes in accordance with the nomenclature adopted³¹ for lactate dehydrogenase and lobster GPDH. The four NAD molecules bind to the enzyme close together near the centre of the molecule. *a*, The enzyme tetramer viewed down the R-axis; the S-loop regions are labelled 'S'. *b*, Viewed down the P axis.

NADH is replaced by NAD (IV, Fig. 5); this accelerates phosphorolysis and promotes specificity for phosphate instead of water as the acyl acceptor³⁵. Model building shows that the attacking phosphate in the transition state (V) can be stabilised by hydrogen bonds with serine 148 and threonine 150, the amide nitrogens of cysteine 149 and threonine 150, and the C2 hydroxyl of the substrate. The C1 oxygen can be protonated by histidine 176. The 3-phosphate remains in the phosphate site P_s. The close proximity of the sulphur of cysteine 149 to the positively charged nicotinamide C4 would explain the acceleration of the reaction by NAD.

'Racker' band

The characteristic absorption band at λ_{max} 360 nm in holo-GPDH, the 'Racker' band³⁶, has been explained by the close association of the sulphhydryl of cysteine 149 and the nicotinamide ring giving rise to a charge transfer complex¹².

We find that the addition of Hg^{2+} to holo-GPDH abolishes the Racker band, but unlike carboxymethylation, it does not weaken the binding of NAD to crystals. The crystal structure shows that mercury binds behind the active site between the SH groups of cysteines 149 and 153, rotating the side chain of cysteine 149 so as to remove the interaction between the SH and the nicotinamide, but it produces no other structural changes. It follows that this interaction can make only a small contribution to the NAD binding energy. The destruction of the Racker band by mercury without any other changes in the environment of the nicotinamide supports the assignment of the band to a charge transfer complex^{37,38}. The distance of at least 3 Å between the SH of cysteine 149 and the nicotinamide ring rules out a covalent interaction³⁶.

Thermostability

B. stearrowthermophilus GPDH retains structural integrity and enzymatic activity at temperatures up to 60–70 °C (refs 39, 40), while the *Thermus aquaticus* enzyme⁴¹ remains stable and active to 95 °C. The additional stabilisation energy needed to produce these dramatically increased thermostabilities amounts to only 5–10 kcal. Attempts to identify the source of increased thermostability by relating amino-acid sequences of the thermophile

GPDHs to molecular models^{41,42} were of limited success owing to the difficulty of distinguishing between sequence changes that are due to phylogeny from those involved in thermal stabilisation. Perutz and Raidt⁴³ compared the structures and sequence of ferredoxin from a mesophile with the sequences of the same enzyme from various thermophiles and found that their greater heat stability arises mainly from external salt bridges linking residues near the amino terminus to others near the carboxy terminus.

Now that the three-dimensional structures of a thermophilic enzyme and its mesophile counterpart have been independently determined it is possible to search for the stereochemical basis of the extra thermal stability. At the level of accuracy so far attained for the structures, the areas of hydrocarbon surface forming buried contacts within the individual subunits are not significantly different in the two enzymes. Changes in the internal hydrophobic residues between lobster and *B. stearrowthermophilus* are complementary and are accommodated without structural change. While the P-axis interfaces are essentially the same in both proteins, the hydrophobic interactions at the R-axis interfaces differ especially among residues 184–187. Several aromatic residues are in contact at the edge of this interface in lobster but not in *B. stearrowthermophilus*. Three tyrosines on the α_c -helix stack with a tryptophan in the S loop of the R-axis related subunit, giving rise to hydrophobic interactions between subunits, not present in the thermophile enzyme, which would indicate greater stability for this region of the lobster enzyme. Contrary to previous speculation, we find the core of the thermophilic enzyme to be no more hydrophobic than that of the mesophilic enzyme.

Although there is a close correspondence between the two structures, a comparative enumeration of hydrogen bonds cannot be made accurately, as their presence can only be inferred from the disposition of hydrogen-bonding groups which are very sensitive to detailed interpretation.

The *B. stearrowthermophilus* enzyme contains more arginines and two of these extra arginines form salt bridges across subunit interfaces. Arginine 194 forms a buried salt bridge with aspartic acid 293 from the P-axis related subunit. Due to the different conformation of the β -sheet in the catalytic domain

this particular aspartate is buried in the thermophile while it is accessible to solvent in the lobster enzyme structure. This salt bridge would not have been predicted simply by relating the sequence of the thermophile enzyme to the molecular model of the lobster enzyme. In addition, arginine 281 of one subunit and arginine 281 from the Q-axis related subunit form a double salt-bridge with glutamic acid 201 from both the R- and P-axis

related subunits. These interactions are shielded from solvent and bring the S loops from two subunits into contact with the other two subunits. These additional inter-subunit salt-bridges evidently stabilise the tetrameric structure of *B. stearrowthermophilus* GPDH, but we would expect that the overall stability of a thermophilic multisubunit enzyme also requires stabilisation of the tertiary structure of the monomer⁴¹. The detailed structural

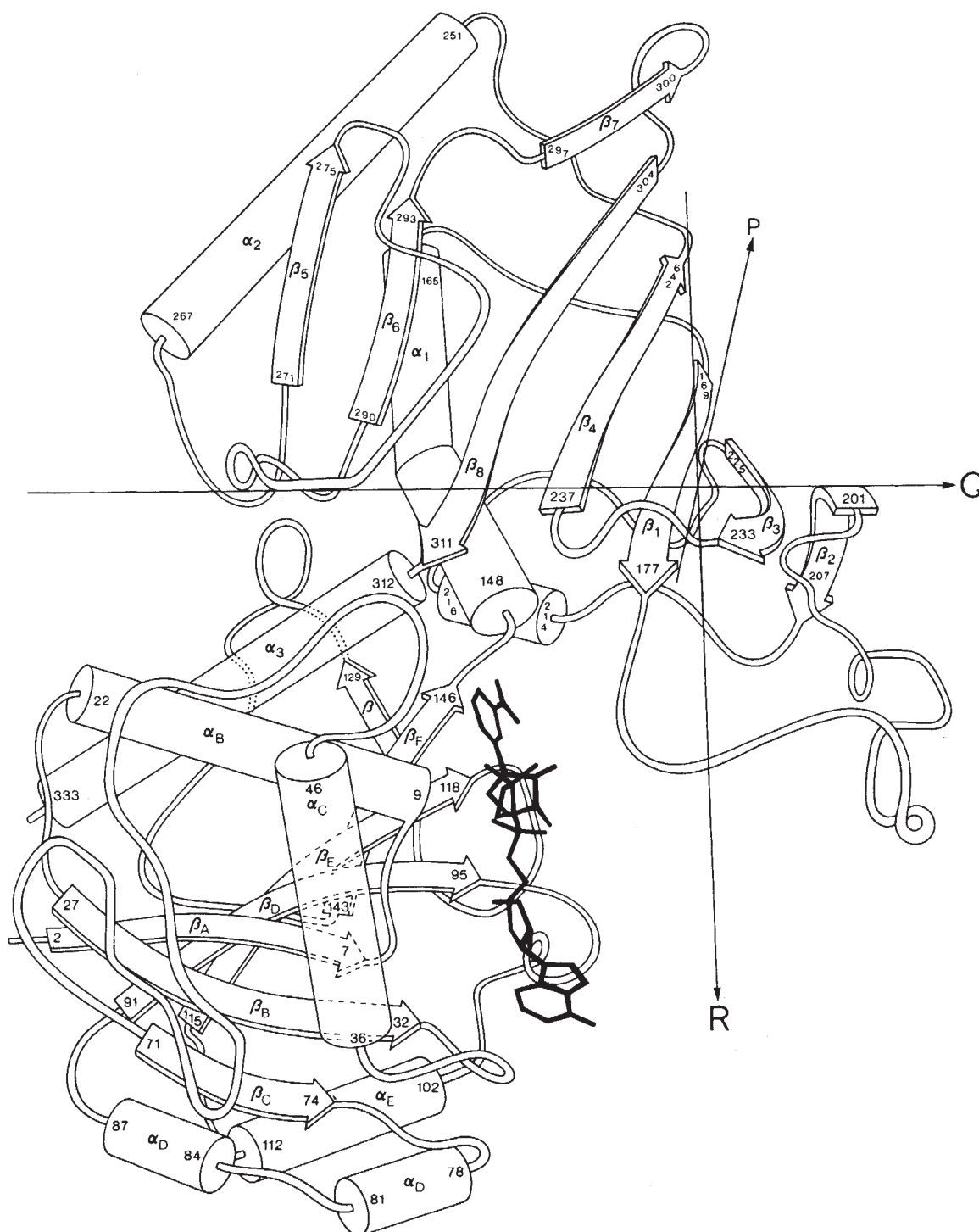


Fig. 3 A folding diagram of one subunit of *B. stearrowthermophilus* GPDH. The nomenclature for the sheets and helices of the first domain is that used for lactate dehydrogenase and lobster GPDH (ref. 31). The folding in the first domain (residues 0–149) is a β - α - β pattern with a central sheet covered on both sides by helices. In the second domain the extensive anti-parallel β -sheet region forms a subunit interface with the equivalent region of the P-axis related subunit. The S loop (residues 178–201) forms the intersubunit contact with the S loop and α_C helix of the subunit generated by the R axis. The carboxy-terminal helix, α_3 , fits into a groove in the first domain. The secondary structure closely resembles that of lobster GPDH except where there are insertions or deletions in external loop regions between segments of secondary structure.

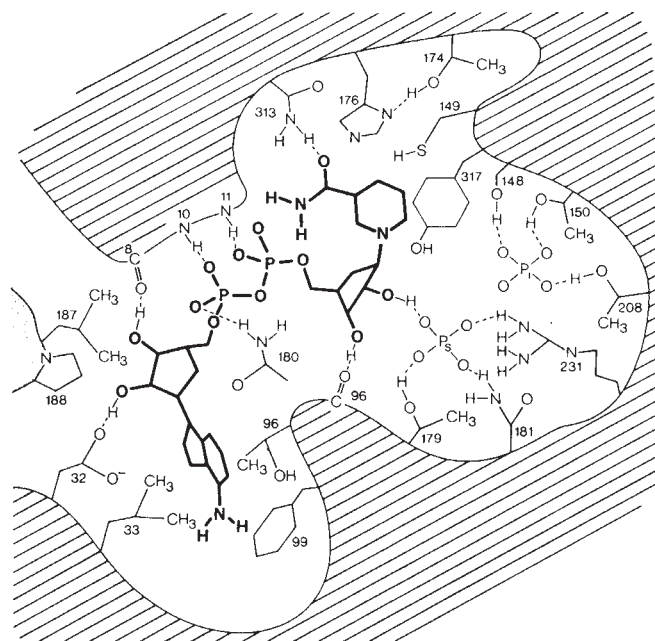
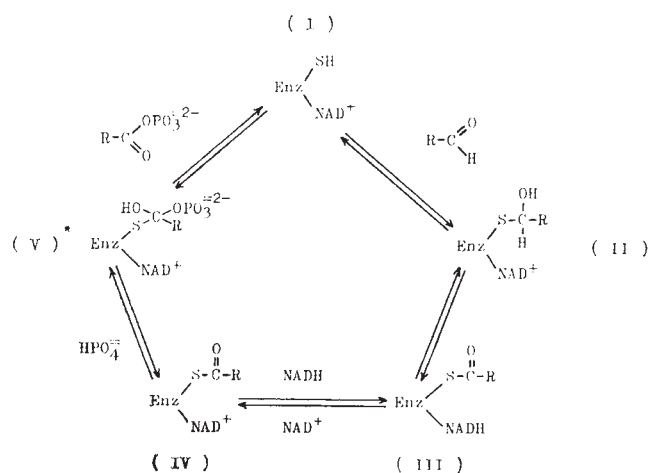


Fig. 4 Residues involved in NAD binding and the two anion binding sites in *B. stearrowtherophilus* holo-GPDH. Residues leucine 187 and proline 188 from the R-axis related subunit are in van der Waals contact with the adenosine ribose. In lobster GPDH phenylalanine 34 replaces leucine 33 on one side of the adenine pocket; proline 188 is conserved but residue 187 is glycine. Asparagine 180 is replaced by alanine in the lobster enzyme. The anion binding sites are labelled P_s and P_i . Model building studies suggest that the site for the 3-PO_4 of the substrate is P_s ; the inorganic phosphate involved in the phosphorylation step would be P_i (ref. 12).

differences, particularly at the enzyme surfaces, make the analysis of the increased stability of the *B. stearrowtherophilus* enzyme extremely complicated since the sum of a large number of stabilising and destabilising effects must be considered. There is no evidence for the involvement of bound cations such as the calcium ions found in thermolysin⁴⁴. Part of the stabilisation energy may be derived from surface hydrogen bonds and salt bridges⁴³, but on present evidence we cannot exclude contributions from other effects.

Fig. 5 The reaction mechanism of GPD (after ref. 33). After the enzyme (I) reacts to form a hemithiacetal (II) a hydride transfer gives an acylhydrazide (III). NADH is replaced by NAD (IV), then phosphorylation via the proposed transition site (V) yields product and free enzyme.



Molecular symmetry

Folding of the main chain is very similar in the lobster and *B. stearrowtherophilus* enzymes but there are differences in the interpretation of the molecular structures. Although the subunits of the fully ligated form of GPDH from *B. stearrowtherophilus* are not related by crystal symmetry, they are nevertheless related by 222 symmetry. The positions and occupancies of five heavy atom sites show the 222 symmetry to be accurate. No structural differences between the four subunits had to be invoked to fit them to the four independently determined electron density images.

An averaged electron density map was not used for interpretation. Starting from a map based on isomorphous replacement with two derivatives, the non-crystallographic symmetry was incorporated to improve the phase angles by the method of Bricogne²³. After two cycles of this procedure a final map was calculated with the observed structure amplitudes and the phases from the final phase recombination⁴⁵. The map obtained by this procedure is much better than it would have been after averaging, which reduces the noise level but also smears out detail, especially of side chains. The advantage of phase combination over averaging has also been demonstrated for hexokinase⁴⁶.

This technique improves primarily those phase angles which are poorly determined by isomorphous replacement, but it is valid only if the molecular symmetry is accurate. For reflections with a figure of merit, $m > 0.7$ from isomorphous replacement (56% of the total), the average phase change after incorporation of molecular symmetry was only 20°. In contrast, the standard error in phase angle expected for $m = 0.8$ is 37°, which shows that the phases from isomorphous replacement are consistent with accurate molecular symmetry.

Moras *et al.*¹² averaged their map of lobster GPDH about the Q dyad and interpreted the map in terms of a non-symmetrical molecule, concluding that NAD is bound differently to different subunits. They have advanced this asymmetry as a partial explanation of the negative cooperativity of NAD binding. In their interpretation the adenine ring of NAD of one subunit is rotated by 180° relative to that of the R-axis related subunit, and the interaction of the adenine ribose with the protein is different in the two subunits. They also suggest that cysteine 149 in one subunit is more closely associated with the nicotinamide ring and, in the R-axis related subunit, more closely with histidine 176. Other differences between the S loops of symmetry related subunits are also noted.

In *B. stearrowtherophilus* GPDH the conformation of the NAD molecule is the same in all four subunits. The four NAD binding sites are equivalent. The sulphhydryl of cysteine 149 lies halfway between the nicotinamide ring and histidine 176 in all four subunits, and there is no density connecting cysteine 149 and the nicotinamide. Histidine 176 is probably hydrogen bonded to threonine 174, although tyrosine 311 is close by, and the orientation of the histidine ring is the same in all subunits. The anion binding sites are also present in all four subunits. None of the asymmetry of subunit structure described for lobster GPDH has been found. In view of the functional and structural similarities of the two holoenzymes it is unlikely that one is symmetric and the other not.

NAD-dependent structural changes

Symmetry of the enzyme is maintained either in saturating concentrations of NAD, or in the absence of NAD. In crystals grown in lower concentrations of NAD, or soaked in NAD-free mother liquor, 0.5–1.5 moles of NAD are lost and structural changes in the protein appear in difference maps against holo-GPDH (ref. 48). The largest changes occur in the nucleotide binding domain of the subunit which has lost its NAD. The conformation of this domain changes towards that observed in a 6-Å map of apo-GPDH (ref. 47).

The large movement of the coenzyme-binding domain on loss of NAD to form apo-GPDH gives rise to an expansion of the molecule. An increase in molecular volume upon loss of NAD

has also been detected by sedimentation equilibrium studies^{48,50}, and by small-angle X-ray scattering^{51,52}.

The negative cooperativity of NAD binding could be the result of a change in packing of parts of the protein chain, especially of the residues in the S-loop. In holo-GPDH this segment of 20 residues is devoid of secondary structure and most of its main chain is inaccessible to solvent. Expansion of the molecule in apo-GPDH opens this region and might make the S-loop more flexible. The following chemical evidence supports this. If muscle apo-GPDH is acylated, and then incubated at pH 8.5, the acyl group is transferred specifically from cysteine 149 to N^ε of lysine 183 (refs. 26, 27). (Residue 183 is arginine in the bacterial enzyme, but the position of the arginine side chain is the same as that of the lysine 183 in lobster GPDH.) In holo-GPDH this transfer does not happen because the N^ε is 10 Å from the sulphhydryl of cysteine 149. The occurrence of the reaction in apo-GPDH only, is indicative of the greater flexibility of the S-loop.

The S-loop has many of the properties expected of a structure which transmits a structural change from one subunit to another in a cooperative system. It interacts with two adjacent subunits (including a direct coenzyme contact), it is shielded from solvent at least in holo-GPDH and lacks strong secondary and tertiary interactions. In addition, there is evidence that its structure in the apoenzyme is quite different from that in the holoenzyme.

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letters to nature

A better way of searching for black-hole explosions?

BLACK holes of $\lesssim 10^{15}$ g evaporate in $\sim 10^{10}$ yr, and eventually annihilate into a burst of energetic photons and particles¹. To test this theoretical prediction would be extraordinarily significant for quantum and gravitational physics. There could be $\sim 10^{23}$ 'miniholes' within our Galaxy^{2–4}; on the other hand, there may not be any at all. But even if they exist in profusion, how best could we detect black-hole explosions? Attention has been focused on the problem of directly detecting the γ rays, but the prospects seem bleak⁵. The possibility that the particles ejected in the explosion may generate conspicuous effects has hitherto been overlooked. I argue here that collective interaction of electrons and positrons with an ambient interstellar magnetic field can (on some specific assumptions) generate radio bursts powerful enough to be detected from anywhere in our Galaxy, or even beyond. This opens up a more promising perspective on the search.

A conducting sphere, expanding into a uniform magnetic field, will develop surface currents which prevent the field from

penetrating it. If the shell expands relativistically, with constant Lorentz factor $\gamma \gg 1$, out to a radius $r_{\max}$, then the work done against the field is

$$\sim \gamma^2 \times (\text{energy of swept-up field}) \quad (1)$$

(Note the factor γ^2 , which occurs just as it would if the sphere were reflecting electromagnetic radiation.) A distant observer would see the part of the shell moving at angles $\lesssim \gamma^{-1}$ to the line of sight blue-shifted by $\sim \gamma$; he sees a surface current on this part of the shell increasing $\propto t^2$ for a time $\sim r_{\max}/c\gamma^2$. The energy (1) therefore appears as an electromagnetic pulse with characteristic wavelength

$$\lambda \simeq r_{\max}/\gamma^2 \quad (2)$$

We now investigate how this classical electromagnetic phenomenon might apply to the type of 'fireball' resulting from an exploding black hole.

Suppose that, when the mass of an evaporating minihole has fallen to M_{crit} g, it vomits forth its remaining energy in an

essentially instantaneous burst of $\sim 10^{21} M_{\text{crit}}$ erg. In the Hagedorn theory, $M_{\text{crit}} \simeq 10^{14}$ g; but on other theories of elementary particles it would be lower. Very near the hole, the energy would be distributed between a variety of exotic species; but we assume that these quickly decay, and that, by the time the ejecta have expanded to macroscopic radii, a substantial fraction (say $\sim 50\%$) of the energy is in a 'fireball' of electron-positron pairs¹⁸. From the theoretical relation between temperature and mass, we expect this fireball to expand with $\gamma_f = (M_{\text{crit}}/2 \times 10^{16} \text{ g})^{-1}$.

For illustrative purposes, we consider fireballs with energy $\epsilon_f \simeq 10^{37} \gamma_f^{-1}$ erg in electron-positron pairs. The number of pairs is then $N_f \simeq 10^{13} \gamma_f^{-2}$.

We must then address these questions:

- (i) How large a radius can the fireball attain before the energy in the swept-up field decelerates it?
- (ii) To what extent is it a good enough conductor to sweep up the field?
- (iii) Would the fireball be braked by sweeping up external plasma as well as magnetic flux?

Suppose that the uniform external magnetic field is $B \simeq 5 \times 10^{-6} b$ G (where b would be ~ 1 for a typical interstellar field with energy density $\sim 10^{-12}$ erg cm⁻³). If the fireball behaved as a perfect conductor, it would be significantly braked when it had swept up a magnetic field energy of $\sim \epsilon_f/\gamma_f^2$. Thus we have, on energetic grounds, an upper limit to r of

$$r_{\text{en}} \simeq (10^{16}/\gamma_f) b^{-2/3} \text{ cm} \quad (3)$$

Quite apart from this limit, the fireball cannot behave like a perfect conductor and expel the magnetic field (without being decelerated) unless the charge density is sufficient to carry the currents. This requires a surface density $\sim 10^3 \gamma_f b$ charges per cm². The conductivity condition thus breaks down at a radius r_{cond} where $N_f/2\pi r^2$ falls to this value. One finds

$$r_{\text{cond}} \simeq (4 \times 10^{19}/\gamma_f^{3/2}) b^{-1/2} \text{ cm} \quad (4)$$

The appropriate value of r_{max} to use in (1) and (2) is the smaller of r_{en} and r_{cond} . For $\gamma_f < 10^7$, r_{cond} exceeds r_{en} (that is, the conductivity is good at all relevant times); so a fraction ~ 1 of the total fireball energy then emerges as electromagnetic waves of characteristic wavelength $\lambda \simeq r_{\text{en}}/\gamma_f^2$. This means that, for $\gamma_f \simeq 10^5$, $\sim 10^{32}$ erg emerges at $\lambda \gtrsim 10 b^{-3}$ cm; and for $\gamma_f \simeq 10^7$, $\sim 10^{30}$ erg emerges at $\sim 10^4 b^{-3}$ Å.

Such a radio burst would be readily detectable anywhere in our Galaxy, or even beyond^{6,7}, and the optical flash from a $\gamma_f \simeq 10^7$ fireball could be detected $\gtrsim 1$ kpc away (J. V. Jelley *et al.*, to be published). We must, however, check the validity of our assumption (question (iii) above) that the fireball is not braked by external plasma before attaining a radius r_{en} . External particles can be swept up, yielding a relativistic blast wave, only if the gyroradius of a particle with Lorentz factor γ_f in a field $\gamma_f B$ is $< r/\gamma_f$. This requires

$$r \gtrsim 4 \times 10^8 b^{-1} \gamma_f \quad (5)$$

In the cases $\gamma_f \simeq 10^5$ and 10^7 cited above, $r_{\text{max}} (= r_{\text{en}})$ does not satisfy (5) for $b \lesssim 1$, so we are justified in assuming that the fireball transfers its momentum to the field rather than the ambient particles. (Arguments analogous to those used in deriving (4) and (5) show that the weak steady efflux preceding the final explosion cannot create a field-free heliosphere-type cavity around a minihole. This vindicates our assumption that the fireball impacts directly on to a typical ambient magnetic field).

In the 'extreme Hagedorn' case ($\gamma_f \simeq 300$), where the earlier argument would have yielded a strong ultra-low-frequency pulse that in any case would not propagate freely, (5) is fulfilled, so we would expect a relativistic blast wave with a swept-up shell. There is then no chance of getting efficient coherent radiation, the energy going instead into random relativistic

particle motions. The field strength in the shell would be $\sim 5 \times 10^{-3} b$ G, and relativistic electrons with $\gamma_f \simeq 300$ would lose only 10^{-10} of their kinetic energy while the shell expanded. If a Hagedorn-type explosion occurred on a timescale Δt sudden enough to cause a coherent radio pulse, then most of the pairs would in any case have annihilated into photons. (The expansion timescale, in the comoving frame, when the fireball temperature falls to ~ 0.5 MeV is $\propto \gamma_f^{-2}(\Delta t)^{1/2}$, and our assumption that there is no time for annihilation to occur is self-consistent for $\gamma_f \gtrsim 10^5$.)

It is thus the case $\gamma_f \gtrsim 10^3$ which—even though it entails a lower ϵ_f —offers the greatest promise of radio (or optical) detection. The pulse-production mechanism requires that the final outburst be 'instantaneous' in the sense that its timescale is $\lesssim \lambda/c$. This requirement is perhaps not met by quark theories, unless a lot of extra species occur above some energy $\gtrsim 100$ GeV. Because of this sensitivity to the uncertain particle physics, the absence of frequent strong radio pulses reaching the Earth cannot be used to improve the present upper limits on the number of miniholes in the Galaxy²⁻⁴. On the other hand, linearly polarised radio-frequency pulses (rather than γ -ray pulses) may well be much the most conspicuous manifestations of black-hole explosions, because radio techniques are exceedingly sensitive^{6,7}. To dramatise the potential advantage, the γ rays from a fireball with $\gamma_f \simeq 10^5$ would only be registered by a detector of effective area 100 cm² if the event occurred within 10^{-2} pc; but even a crude non-directional antenna would detect the corresponding radio pulse at distances $\sim 10^4$ pc. And an Arecibo-type telescope could detect such pulses—each triggered by a single entity of subnuclear size—from as far away as the Andromeda galaxy!

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Is Cas A illuminated by a binary system?

I REPORT here indications that the supernova remnant Cas A (and probably many others) contains a neutron star at its centre, in close orbit around a 'normal' star. The indications are of three types: of evolutionary, statistical and indirect character, arguing that the high observed magnetic field strengths and the steepening synchrotron spectrum cannot be explained by stochastic processes.

Supernova explosions are commonly believed to derive their energy from the collapse of the (degenerate) core of a formerly massive late star. The conversion of gravitational energy into the kinetic energy of radial motion could be achieved through a magnetic spring¹⁻³ rather than a combination of neutrino⁴ and thermal pressures. The mass of the collapsing core is expected to be near the Chandrasekhar mass ($\approx 1.4 M_{\odot}$), because this is the minimum mass for gravitational instability according to stellar evolution theory, and because all the overlying material is ejected by the action of the magnetic spring. The core thus turns into a stable neutron star. Mass determinations⁵ of neutron stars in binary systems confirm this picture, which might apply to supernovae of all types (I, II, ...), their differences being due to the density of the surrounding medium which controls the mass in the envelope.

Most if not all of the massive progenitor stars of supernovae occur in binary (or multiple) associations. During the explosion, a binary system gets disrupted if the ejected mass is bigger than the remaining mass, which can happen in the first or, more likely, in the second event. If in the first event, we obtain (eventually) two pulsars; if in the second, mass flow from the normal star to the neutron star can give rise to a temporary X-ray source, and eventually to one or two pulsars (depending on the spin rate of the first neutron star). The measured peculiar velocities⁶ of 12 pulsars suggest that several of them received two kicks in the past, and hence result from binary systems containing a neutron star.

We therefore expect a large number of supernova explosions to leave a binary neutron star at their centre. What will such a system look like? The young neutron star should act as a strong pulsar, with a relativistic wind of power $L \approx 10^{39} \text{ erg s}^{-1}$ ($\Omega_{2.5}^4$ (modelled after the Crab). A fraction of this power will heat the atmosphere of the companion star until enough plasma is evaporated for the formation of a common envelope. The time scale t for evaporating a small fraction α of the companion star's mass M is of the order (ignoring reradiation)

$$t = \alpha GM^2 a^2 / R^3 L \approx 30 \alpha \alpha_{-3} a_{12}^2$$

where a is binary separation and R the stellar radius. For close binaries, a small evaporated mass fraction α should be sufficient to build up an atmospheric pressure which overcomes the pulsar wind pressure, first at mid-separation, and later at all distances outside a small cavity around the neutron star, so that a common envelope forms, and the pulsar is quenched. Instead of driving a relativistic wind, the rotating magnetised neutron star will henceforth accelerate the wind plasma of the companion star which impinges on its magnetosphere (through magnetospheric friction), and produce cosmic rays⁷. It will no longer act as a pulsar. Cyg X-3 could be a system in transition between the pulsar stage and the cosmic-ray stage, whereas Cas A is suggested to have reached the latter.

To obtain an independent estimate of the duration t of the pulsar stage, and to assure consistency of the whole model, we consider statistics. The present birth rate of supernovae in the Galaxy has been estimated⁸ to lie between 10^{-1} and 10^{-2} yr^{-1} . Allowing for an enhanced activity throughout an early phase of the Galaxy, the total number of supernova explosions since formation should be 10^8 to $\geq 10^9$. Unless neutron stars can be formed in a peaceful way^{9,10}—which is not very likely even when magnetic fields are ignored—this number must not be smaller than the total number of neutron stars in the Galaxy. Neutron stars occur singly, as pulsars, and in binaries, visible during the X-ray stage. Extrapolation of the known data^{11–13} on pulsars leads to a present total number between 10^4 and 7×10^5 , depending on the distance scale, and upon the amount of beaming into a reduced spherical angle. Pulsar lifetimes peak near $3 \times 10^6 \text{ yr}$ so that there should be (at least) a total of 2×10^7 to 2×10^9 single neutron stars (= dead or alive pulsars) in the Galaxy. (Single neutron stars ejected as slow rotators from binary systems are not included.) This suggests a one-to-one correspondence between supernova explosions, and neutron star formations; Cas A may not form an exception.

Next, a single pulsar has not been seen in orbit around a normal star, although there should be^{14,15} over 10^4 such systems in the Galaxy (inferred from the number of X-ray sources, or from the number of pulsars.) This implies that the pulsar stage t must be $\lesssim 10^{-4}$ times shorter than the lifetime of a massive star, that is, shorter than some $5 \times 10^2 \text{ yr}$.

Cas A has an estimated age¹⁶ of $3 \times 10^2 \text{ yr}$, and a radius of 5 light yr. It does not contain a pulsar: even if the pulses were beamed away from us, we should see strong optical emission from the inner edge of the nebula, like the wisps in the Crab, or at least a strong compact X-ray source. Also, pulsar peculiar velocities¹⁷ are rarely higher than $10^{-3} c$ so that a pulsar could not have escaped from the nebula. For these reasons, Cas A

has been held to be a remnant without a central source, and has been treated¹⁸ as a hot cloud expanding due to a powerful explosion in the past. Such a detonation wave becomes unstable, near its outer edge, towards convective motion and mixing with the interstellar plasma. From his numerical models, Gull¹⁸ estimates a conversion of 2 or 3% of the total expansion energy into convection, which is held responsible for driving a dynamo that enlarges the interstellar magnetic field by a factor of $\sim 10^2$, to arrive at the inferred equipartition field strength of $5 \times 10^{-4} \text{ G}$ in the outer parts of the remnant. In most of the compact knots, the minimum magnetic field inferred from the radio synchrotron brightness is as high as $3 \times 10^{-3} \text{ G}$. The radio intensity decreases with time, by roughly 1% per yr, but at a higher rate (1.3%) at 85 MHz than at 1.4 GHz (0.9%). Moreover, there is a soft X-ray luminosity of $3 \times 10^{36} \text{ erg s}^{-1}$.

The difficulty faced by this model is the large observed magnetic field, which would require (many) more than seven turns to be performed by the interstellar material; even if there is enough energy available, a dynamo cannot do better than double the seed field during each turnover. With the observed convective velocities $\lesssim 10^{-2} c$ (ref. 18), such a dynamo cannot reach scales beyond 10^{-1} light yr. Similar difficulties arise for the radiating electrons whose synchrotron lifetimes are shorter, at energies above 10^2 GeV , than the lifetime of the remnant, and also much shorter than the replenishment timescale for Fermi acceleration¹⁹.

These difficulties disappear if we assume the existence of a neutron star inside the remnant. This neutron star would have acted as a pulsar for an initial period of the order of some 10^2 yr , during which its extremely relativistic wind injects both electrons and magnetic flux into the supernova shell²⁰. When overtaking trailing filaments (protostellar condensations), the frozen-in magnetic field gets hooked up, and forms the magnetic knots—which I interpret as filaments seen edge on. (Such knots do not occur in the Crab nebula, whose symmetry axis is nearly normal to the line-of-sight). For the same reason, the field line geometry between knots is near-radial.

The main reasons against an empty interior of Cas A are therefore, in decreasing order of cogency: (1) The finite scale, strong magnetic fields are difficult to explain, because a dynamo needs time; and relativistic particles are difficult to produce by the more or less ordered (radial) motion of a detonation wave. (2) If the majority of supernova explosions did not result in the birth of a neutron star, we might see more pulsars than have been (violently) born. (3) There is a trend both in late stellar evolution theory, and in astration theory²¹ towards assigning an increasingly large probability to neutron star formation in supernova explosions.

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Dirac-Fock one-centre calculations show (114)H₄ to resemble PbH₄

RELATIVISTIC effects have a considerable influence on the chemical behaviour of the heavier elements. Our Dirac-Fock one-centre expansion calculations¹⁻³ indicate that the X-H bond lengths, R_e , suffer a relativistic contraction of about 5% for PbH₄ (ref. 1) or AuH (ref. 2) and 7% for TIH (ref. 3). If the relativistic effects are included, however, these R_e values mostly agree with experiment to within 2-3%. Relativistic effects also seem to be the main cause of the chemical difference between silver and gold² and, possibly, of the dominant monovalency of thallium³. A relativistic treatment becomes imperative to predict the chemical behaviour of the superheavy elements. Following evidence for primordial superheavy elements⁴, we present here results for the tetrahydride of the element 114 or eka-lead. We believe that such a calculation gives a reasonable estimate for the covalent radius of this element. Although the dominant valency of 114 is expected to be 2, some tetravalent compounds are also expected to be stable⁷. As the latter valency is more convenient for calculations, having closed shells, we here consider (114)H₄.

Our approach here is as in ref. 1. No symmetry functions were used. A finite, homogeneously charged nucleus with $A = 298$ was assumed. The total energy, E_T , was calculated at five internuclear distances and the results were fitted into a third degree polynomial. The results are shown in Table 1, E_T

Table 1 Calculated results for (114)H₄

Bond length R_e	3.376 AU = 1.787 Å
Total energy $E_T(R_e)$	-49712.76 AU
Force constant k_2	0.57 AU
Orbital energies ϵ^*	
7 $p_{3/2}$	-0.25712 AU
7 $p_{1/2}$	-0.43640
7 $s_{1/2}$	-0.80274
6 $d_{5/2}$	-0.77015
6 $d_{3/2}$	-0.93950

*At $R = 3.4$ AU

variation in Fig. 1, and orbital energies in Fig. 2. The most striking aspect of the Fig. 2 is that the 6 $d_{5/2}$ shell is slightly above the 7 s . Therefore, a strong participation in chemical bonding is expected from the 6 $d_{5/2}$ and 6 $d_{3/2}$ shells. Similarly, a strong participation of the 5 d orbitals is suspected for AuH (ref. 2). The present values of R_e and k_2 , 3.376 and 0.57 AU, respectively, are closely similar to those obtained¹ for PbH₄ using the same approximations, or 3.395 and 0.55 AU, respectively. The experimental k_2 for both PbH(CH₃)₃ and PbH₂(CH₃)₂ is 0.445 AU (ref. 9).

The covalent radii for elements C to Pb (ref. 6) are listed in the first column of Table 2. Combining them with the experimental R_e values in the second column, we find a covalent radius of 0.31 Å for hydrogen. Subtracting this value from the present theoretical R_e of 1.787 Å we find a covalent radius of 1.48 Å for quadrivalent (114). The difference of 0.04 Å between the R_{cov} for Pb and 114 includes the experimental error for PbH₄. The theoretical R_e differs by 0.01 Å.

As the effects of increasing atomic number, Z , on the bond length are quite different for Groups IB (Au), IIIA (Tl) and IVA (Pb), it is interesting to discuss these differences in terms of the electron configurations. Group IB has an s^1 valence configuration which is also the dominant component of the bonding molecular orbital in AuH. As this atomic orbital contracts strongly⁵, due to relativistic effects, the experimental R_e for AuH is 0.09 Å shorter than that for AgH. For Group IIIA the valence configuration is p^1 . According to the model in ref. 3, a transition from $p\sigma$ bonding to $p_{1/2}$ bonding takes place when going from InH to TIH. Therefore the lost directional character

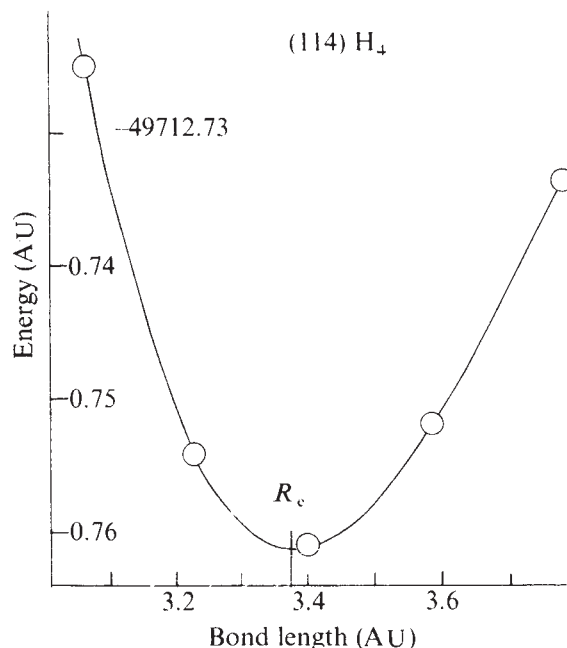


Fig. 1 Molecular total energy as a function of bond length. The curve is the fitted third degree polynomial.

makes the chemical bond of TIH weaker. Although the $p_{1/2}$ shell itself contracts, the sum of the two effects makes the R_e for TIH 0.02 Å larger than for InH. For the tetrahydrides of Group IVA, the $p_{3/2}$ and $p_{1/2}$ shells are filled. The average orbital energy $(2\epsilon_{p_{3/2}} + \epsilon_{p_{1/2}})/3$ depends only slightly on Z (Fig. 2). The theoretical R_e values increase by 0.03 Å from SnH₄ to PbH₄ and decrease by 0.01 Å from PbH₄ to (114)H₄. No evident interpretation can be given for these small changes.

We give here the first calculation of a bond length for a superheavy element. The result for the covalent radius of 114 is about 1.48 Å. Assuming also for metallic 114 the same crystal structure and lattice parameter as for Pb, the assumed atomic weight of 298 would lead to a density of 16.3 g cm⁻³ for the metal 114. The general chemical conclusion is that the covalent radii of 114 and its neighbours are expected to be comparable with,

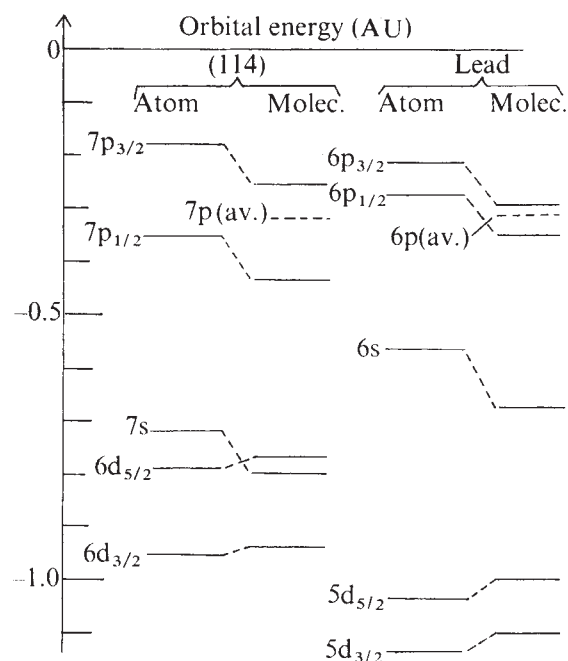


Fig. 2 Orbital energy diagrams for the free atoms of lead and the element 114 (from ref. 5) and for the molecules PbH₄ (from ref. 1, $R = 3.50$) and (114)H₄ (present work, $R = 3.40$).

Table 2 Covalent radii R_{cov} (Å) for the tetravalent elements C to (114), measured X-H distances, $R_{\text{e,exp}}$, for the compounds XH_4 , and the corresponding distances, $R_{\text{e,calc}}$, calculated using R_{cov} .

Element	R_{cov}	$R_{\text{e,exp}}^\dagger$	$R_{\text{e,calc}}$
C	0.77*	1.093	1.08
Si	1.17*	1.480	1.48
Ge	1.22*	1.527	1.53
Sn	1.40*	1.701	1.71
Pb	1.44*	(1.754)§	1.75
114	1.48†	—	—

*From ref. 6.

†Present work.

‡From ref. 8.

§Deduced from $R_{\text{e}}(\text{PbH}_4) = R_{\text{e}}(\text{PbH}) + R_{\text{e}}(\text{SnH}_4) - R_{\text{e}}(\text{SnH})$.

or slightly shorter than those of lead and its neighbours. The same is true of the force constants. Unfortunately, we cannot draw conclusions about the chemical stabilities as it is known that, for example, PbH has nearly the same R_{e} and k_2 as SnH but only one half of the dissociation energy¹⁰.

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A melting criterion based on the dilatation dependence of shear moduli

THE elastic instability theory of melting, proposed by Max Born¹, held that melting in a crystalline material occurs when one of the elastic shear moduli vanishes, so that the material can no longer sustain an infinitesimal shear stress without flowing. This explains the fluidity of a melt, but fails to account for several other important characteristics of melting. Melting is a transition between two independent phases, while Born's theory is a single-phase theory which contains no distinct description of the melt, and thus fails to account for the discontinuous, first order character of melting. Because it is an essentially homogeneous theory, it does not explain the occurrence of superheating, the metastability of the melt, and the heterogeneous nucleation and growth features of the melting process. Also, subsequent observations of elastic moduli showed that none of the shear moduli is zero or near zero in the solid at the melting point. Because of these defects Born's instability theory currently enjoys little credibility. We believe, however, that correct application of Born's hypothesis leads to a simple modification of his initial criterion for melting which satisfies all the requirements of a physically realistic theory of melting. We show here that the hypothesis does predict a first-order transition between two phases and that the transition has a nucleation barrier, thus dismissing some of the physical objections to the hypothesis. Moreover, the hypothesis applies in the alkali halides, in that in each case one of the shear moduli falls continuously to zero through the melting expansion.

Suppose that one of the elastic shear moduli, μ , decreases monotonically with temperature increase, and that at some

critical temperature the shear modulus vanishes or becomes sufficiently small that shear flow could occur by thermal activation. The consequent fluidity which allows each atom access to the entire fluid volume will cause an increase in entropy, due to the communal entropy, which in the case of the alkali halides equals $2R$. The onset of fluidity thus results in a decrease in free energy by $2RT$. In the virtual process of tracing back along the shear modulus curve, initially increasing the free energy by losing the communal entropy but progressively decreasing the elastic internal energy, a point can be located, at which the free energy of the solid equals the free energy of the virtual solid that has a vanishing shear modulus. (The elastic internal energy will be decreased by $> 2RT$, as the virtual process results in an additional decrease in vibrational entropy.) Thermodynamically, it is possible for the solid at the point thus located to transform isothermally to the vanishing shear modulus state. This is the melting point, and the transition will be discontinuous with an accompanying heat absorption. We therefore suggest that Born's melting criterion that melting occurs when the shear modulus of the solid vanishes, should be replaced by the following: melting occurs when a solid can transform isothermally to a state of zero shear modulus. The modified theory becomes a two-phase theory in that the solid at the melting point is described as solid at dilatation δ_m^s while the melt at the melting point is described as virtual solid with properties evaluated at $\delta = \delta_m^l$. Moreover, there is a nucleation barrier to melting equal to the communal entropy, and consequently the heterogeneous character of melting is also implicated by the hypothesis.

The behaviour of the alkali halides agrees with our proposal. Fig. 1 shows a plot of shear modulus, $\frac{1}{2}(c_{11} - c_{12})$ for the

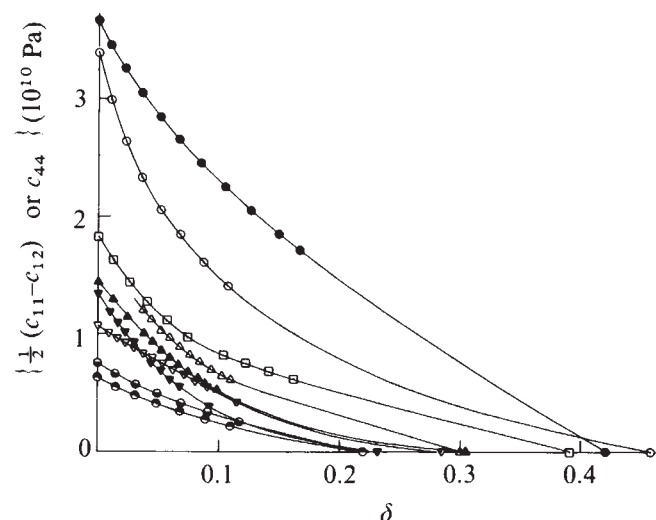


Fig. 1 The shear moduli $\frac{1}{2}(c_{11} - c_{12})$ for some rock salt alkali halides and c_{44} for CsBr and CsI as functions of dilatation, $\delta = \Delta V/V$. $\circ$, LiF; $\bullet$, NaF; $\square$, NaCl; $\triangle$, KCl; $\blacktriangle$, KBr; ∇ , KI; $\blacktriangledown$, RbI; $\bullet$, CsBr; $\bullet$, CsI.

rock salt alkali halides, and c_{44} for CsBr and CsI as a function not of temperature, but of dilatation, that is, the relative increase in volume $\Delta V/V$ from room temperature to the temperature and phase of interest. Our data are obtained from refs 2-5. The last point in the close succession of points on the low dilatation side is the shear modulus at the dilatation, δ_m^s of the solid close to the melting point, while the isolated points for each curve are for $\mu = 0$ at the dilatation, δ_m^l of the liquid at the melting point. Although the shear modulus and the dilatation are both discontinuous with respect to temperature at the melting point, the points for the melt in Fig. 1 lie on the extrapolated curves for the solid, and consequently the shear modulus seems to be a continuous function of dilatation through the melting expansion and falls to zero at the dilatation of the melt. Born's melting hypothesis as modified above thus seems to apply for the alkali halides.

A fuller account of our work and evidence for the continuity of the bulk modulus with respect to dilatation through the melting expansion are presented elsewhere^{6,7}.

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Non-radiative energy transfer in polymers

THE study of intra- and intermolecular transfer of energy by non-radiative processes is important in developing an understanding of such phenomena as photosynthesis and photo-stabilisation of polymers. Several theories have been proposed, notable among which is the dipole-dipole mechanism due to Förster¹ and Galanin². Apart from the dependence on distance separating donor and acceptor, the probability of transfer is related to the angle between donor and acceptor moments. For this reason measurements of fluorescence depolarisation can be used to estimate the efficiency of transfer from donor to acceptor. Macromolecules provide interesting potential for studying intra- as well as intermolecular non-radiative transfer. Thus chromophore-bearing polymers such as polystyrene allow the possibility of excitation at one part of the molecule, transfer along the chain from one phenyl unit to another until an energy trap, an excimer site, is reached whereupon the energy is emitted as a photon. The principal emission observed from polystyrene at room temperature is that from excimer. We wish to make a preliminary report on some studies we have made on fluorescence depolarisation using a styrene-phenyl acetylene copolymer as donor, and perylene as acceptor.

The donor-acceptor pair were cast in poly(methyl methacrylate) films. This copolymer has polyene groups along the main chain as well as the pendant phenyl units. It has a much stronger emission spectrum than polystyrene and is thus more suitable for using in energy transfer studies. The results shown in Table 1 indicate that energy is being transferred from donor to acceptor, at the highest concentration of acceptor transfer is almost complete. At this level of concentration of acceptor the process of transfer is long range and the mechanism must be by dipole-dipole interaction. Applying Förster theory¹ to the data yields an R_0 value of 78 nm indicating coupling over relatively long donor-acceptor distances. Column 2 of Table 1 shows that as the efficiency of transfer increases, the extent of depolarisation of the emission from the acceptor also increases, in accord with the predictions of Förster's theory. It is also worth noting that intermolecular energy transfer is occurring involving perylene molecules only (column 4). However, the emission from the macromolecular donor shows no depolarisation which suggests that neither inter- or intramolecular non-radiative energy transfer is taking place.

We also measured the depolarisation of the excimer emission of several samples of pure polystyrene films cast from methylene dichloride solutions and obtained a value of 1.3 ($\lambda_{ex}=264$ nm, $\lambda_{em}=320$ nm). The instrument used to

Table 1 Depolarisation of styrene/phenyl acetylene copolymer (donor) and perylene (acceptor), suspended in poly(methyl methacrylate)

Acceptor concentration (M)	Efficiency of transfer from donor to acceptor	Depolarisation (1)	Depolarisation (2)	Depolarisation (3)
0	—	—	—	1.8
0.55×10^{-3}	0.40	6.8	3.2	1.7
1.46×10^{-3}	0.59	10.3	3.4	1.6
3.65×10^{-3}	0.73	16.0	4.7	1.5
7.30×10^{-3}	0.81	22.7	5.8	1.9
18.30×10^{-3}	0.99	25.0	10.5	1.9

The donor contained approximately 20% (by weight) of phenyl acetylene, and its concentration was constant at 10^{-3} M.

Depolarisation ($\bar{P}$) was measured using the equation³ $\bar{P} = \frac{I_{VV} + GI_{VH}}{I_{VV} - GI_{VH}}$

Depolarisation (1) was obtained by exciting the donor and measuring the depolarisation of the acceptor emission: $\lambda_{ex}=285$ nm; $\lambda_{em}=442$ nm. Depolarisation (2) was obtained by exciting the acceptor and measuring the depolarisation of the acceptor emission: $\lambda_{ex}=410$ nm; $\lambda_{em}=472$ nm. Depolarisation (3) was obtained by exciting the donor and measuring the depolarisation of the donor emission: $\lambda_{ex}=285$ nm; $\lambda_{em}=380$ nm.

make the measurements was a Perkin Elmer MPF2, using the polarisation accessories provided by the makers. The value obtained for fluorescein was in agreement with the literature value.

The results for the polymers have two possible interpretations: either (1) intramolecular energy transfer for these polymers does not occur to any significant extent by the dipole-dipole mechanism and therefore the currently held theory of energy migrating along the polymer chain into traps is incorrect, or (2) the probability of transfer does not decrease as $\cos^2$ (the angle between moments) but at some higher power. The data presented in Table 1 show that intermolecular transfer is occurring for perylene and consequently the $\cos^2$ relationship holds.

We conclude that energy transfer along polymer chains does not take place by the Förster-type non-radiative mechanism. Further experiments are being carried out to attempt to elucidate the mechanism of intramolecular transfer for polymers.

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Large ground deformation on Mount Etna volcano

AN 11-km traverse of benchmarks for precise levelling across the summit of Mount Etna was established and levelled in July 1975. The traverse runs from a reference benchmark near the Piccolo Rifugio (about 2515 m a.s.l.) in the south, across the summit cone (highest station about 3210 m a.s.l.) and down to the rim of the Cratere Ellittico Caldera near Pizza Deneri (about 2780 m a.s.l.) (Fig. 1). The traverse is re-occupied about twice a year to monitor vertical ground movements associated with volcanic activity, the method being similar to that used on Kilauea Volcano, Hawaii¹⁻³. A Zeiss Ni2 self-levelling level with micrometer and 3-m invar staff are used and precise levelling techniques adopted where possible. (Previous precise levelling on Etna⁴ was restricted to dry tilt stations at three localities.) Closures on $\frac{1}{2}$ -km traverses are better than first order accuracy. Re-levelling has so far been carried out in May 1976 and

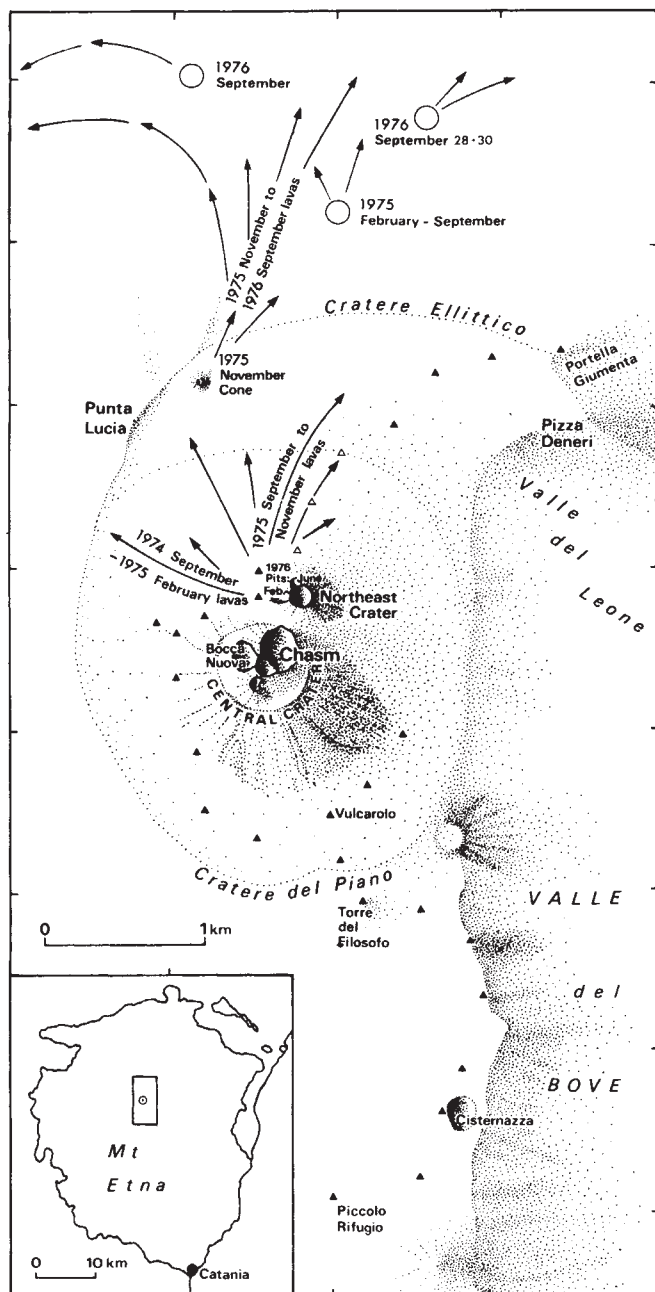


Fig. 1 Map of Mt Etna summit, showing the activity 1974-76, and the location of the levelling traverse. $\blacktriangle$, Principal benchmark; $\triangle$, principal benchmark destroyed by lavas of September to November 1975; $\circ$, approximate centre of quiet emission of lava; arrows show directions of lava flows.

September 1976, and takes about 6 d for the complete traverse. We report here the results of this repeated precise levelling, which show that an area at least 2 km across subsided >1 m between July 1975 and September 1976. This could indicate high-level removal of magma, or incipient caldera collapse.

After being dormant for $3\frac{1}{2}$ yr following the 1971 eruption, the North-east Crater started activity again on 28 September 1974, with strombolian activity quickly followed by lava eruption. This activity ceased on 23-24 February 1975, when lava began erupting from new boccas 2 km to the north at about 2,500 m a.s.l. (Fig. 1). The rate of eruption was measured up to $1.5 \text{ m}^3 \text{ s}^{-1}$ during this phase of eruption, which lasted until 12 September. During the first occupation of the traverse (30 July-5 August 1975) lava was still coming out intermittently at this site, occasionally ceasing for a few days, when minor strombolian activity and internal

collapse occurred at the North-east Crater. This alternation between emission of lava on the north flank and activity at the North-east Crater continued until 12 September, when the north flank effusion stopped, and lava accompanied by strombolian activity erupted from the North-east Crater and began new flows down the northern slopes. These buried >1 km of the northern jeep track, and destroyed 14 of our benchmarks. Rates of eruption of $0.9 \text{ m}^3 \text{ s}^{-1}$ were measured. This activity stopped on 28 November 1975, and on 29 November a new fissure opened 450 m ENE of Punta Lucia (altitude ~ 2900 m a.s.l.), with eruption of lava and violent strombolian activity, which rapidly built up a cone about 40 m high. In early 1976, a collapse pit about 120×70 m formed on the west flank of the North-east Crater, accompanied by considerable emission of ash and the onset of intense sulphur-bearing fumarolic activity.

At the time of the second occupation of the traverse, 20-26 May 1976, activity near Punta Lucia continued with intermittent weak strombolian activity in the cone and emission of lava at a rate of $1-3 \text{ m}^3 \text{ s}^{-1}$. Three weeks after the levelling on 17 June, a new summit vent opened on the north-west flank of the North-east Crater, and strombolian activity ensued, which, assisted by some collapse, produced a crater about 80 m wide. Strombolian activity continued until 20 August, interspersed with a 3-week period of high-pressure gas emission in late July. Quiet effusion of lava near the Punta Lucia vent followed, but by the time the traverse was levelled for the third time (16-21 September 1976) large flows were emerging at a fairly high rate ($3-4 \text{ m}^3 \text{ s}^{-1}$) much further down the northern side, >3 km north of the summit at an altitude of about 2,300-2,400 m.

In May 1976, unusually late snowdrifts prevented re-levelling of the northern half of the traverse, apart from the final 500 m. Vertical movements in this and the southern section proved small, but two isolated benchmarks 40 m apart protruding above the snow 300 m west of the North-east Crater were re-levelled, and gave 3.5 cm of relative downward movement towards the north-east.

In September 1976, new benchmarks were set up on the September-November 1975 lava, so that the whole traverse could be re-levelled. Changes in altitude since May 1976 were again small in the southern section, -1.3 cm being the largest movement; but in the northern section, inacces-

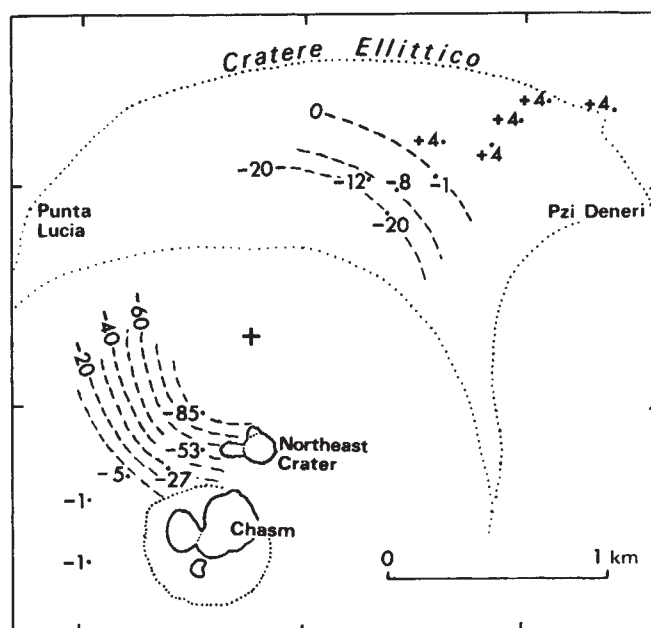


Fig. 2 Map showing relative changes in altitude (cm) of benchmarks between July 1975 and September 1976. ---, Inferred 10-cm contours of altitude change. +, The presumed centre of collapse.

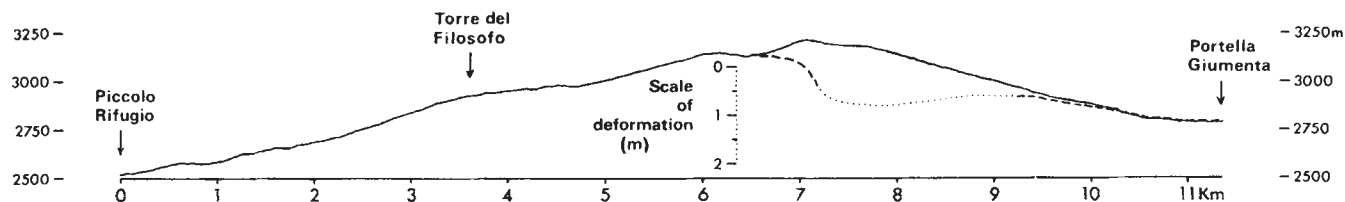


Fig. 3 —, section of the levelling traverse (vertical exaggeration $\times 2$); ---, measured ground deformation between July 1975 and September 1976 with $\times 500$ exaggeration, relative to the profile.

sible in May, the changes since July 1975 are very large. They indicate collapse of an area >2 km wide, from just north-east of the Central Crater in the south, to just south of the Cratere Ellittico in the north (Fig. 2). On the southern side of this area of deflation, the ground deformation increases northwards from -0.9 to -84.6 cm in only 700 m, and assuming a fairly smooth profile of deformation (Fig. 3) the amount of subsidence at the centre of collapse is likely to be >1 m. The maximum amount of collapse cannot be determined exactly since the centre of collapse lies in the unmonitored area where our benchmarks were destroyed. To the north of the unmonitored area, the amount of ground collapse decreases northwards from -19.5 to $+4$ cm, at which slightly inflated value it remains almost steady over the most northerly 800 m of the traverse.

About 1 m of vertical deformation in 1 yr is large, considering that no major eruption took place during this time. The most unusual feature of this subsidence is the small diameter (just over 2 km) of the area affected. This is unlike inflation and deflation on Hawaii, where the whole volcano is affected¹⁻³. The actual tilt of the ground implied by these measurements is at least $1,200 \mu\text{rad}$ on the south side of the area of collapse.

The large amount of vertical deformation compared with the small horizontal extent of the area affected suggests that the cause of the deformation is at a shallow depth, and could result from several causes: one is that as the area of subsidence contains the North-east Crater flows of 1966–71 and 1974–75, we are observing cooling contraction and compaction of these flows. We think this unlikely especially as the 1971 flows on the south side of the summit cone show no such movement. Also, precise level tilt stations on older flows outside the area indicate deflation. Another more likely explanation is that the collapse could be directly related to the summit activity of 1975 September–November and 1976 June–August. One might expect the summit to be inflated in late July 1975, 6 weeks before summit activity, and deflated in September 1976, 1 month after summit activity. The levelling of the two isolated summit benchmarks in May 1976 shows that most of the collapse had already taken place at that time. This is likely to be the case since the summit activity in August was relatively minor. Finally, we could be witnessing incipient caldera formation, and that collapse could continue to take place in the area. Caldera formation has occurred on Etna at intervals of several hundred years, and the recent occurrence of other types of collapse at the summit, such as the collapse of the Bocca Nuova since 1970 and the collapse of the two new pits on the North-east Crater in early 1976 and June 1976 could support this view. The total void created by these three events is about $5 \times 10^6 \text{ m}^3$, added to an estimated $1 \times 10^6 \text{ m}^3$ created by the ground deformation. The volume of lava erupted during this time is at least an order of magnitude larger.

Whichever of the above possibilities proves to be correct, the interest of these results and the importance of continuing these observations is self-evident. They contribute to a greater understanding of the internal structure and workings of the volcano, which will hopefully lead to an increasing ability to predict activity, and point to areas of interest for other studies on the mountain.

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Evidence for a low velocity layer at the base of the oceanic crust

As the quantity of seismic refraction data in the oceans increases it is becoming apparent¹⁻³ that there is a systematic increase in crustal thickness with age, at least out to about 60 Myr (refs 1, 2). To explain this phenomenon more precisely the detailed structure of the lower crust is required. We present seismic refraction data from the Northern Cocos plate which shows that thickening occurs by the formation of a low velocity zone (LVZ) at the base of the crust with a velocity

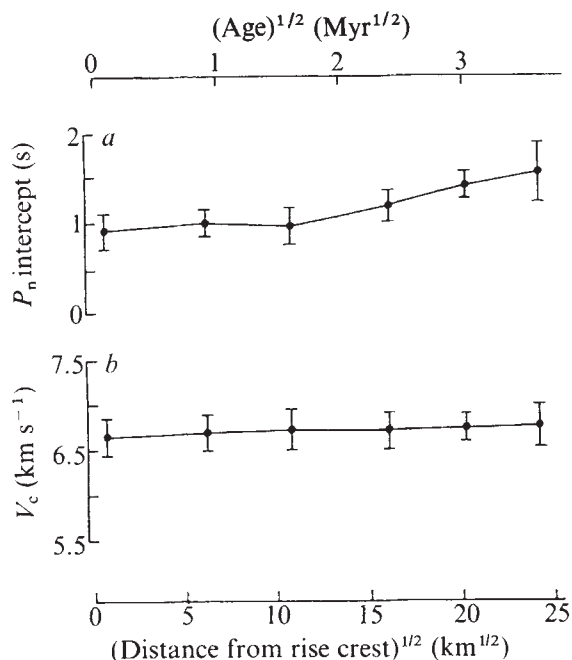


Fig. 1 a, Average mantle intercept times (P_n) and b, average crustal velocities V_c (ref. 2) against $(\text{age})^{1/2}$ in $(\text{Myr})^{1/2}$. The spreading rate is about 4.5 cm yr^{-1} and the error bars represent 1 s.d. The intercept times have had the travel time through the water column subtracted.

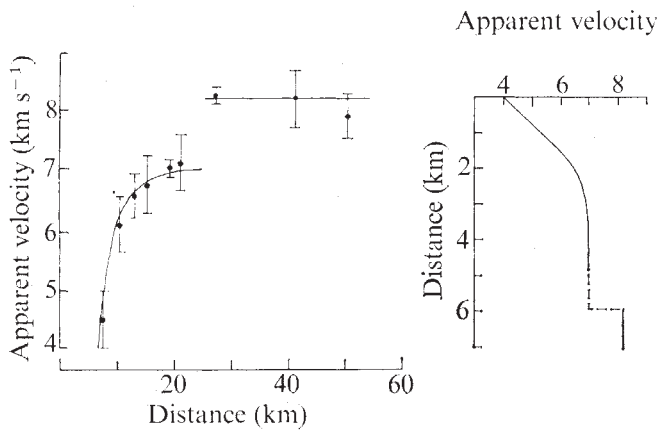


Fig. 2 Apparent velocities of first arrivals (with 1 s.d. error bars) measured across the array of receiving buoys (line 67).

$< 6.8 \text{ km s}^{-1}$. One possible explanation of this in terms of these relatively low seismic velocities for the lower crust⁴, is that upper mantle material becomes serpentinised by a hydration reaction as it cools. If so, this implies that the Moho boundary, except at very young ages, is a phase change (hydration) boundary. This supports the ideas of Hess⁵.

Our data were taken over the Northern Cocos plate in an area bounded on the north and south by the Orozco and Clipperton fracture zones and on the east and west by the Middle American Trench and the East-Pacific rise (EPR). The spreading rate in this area is about 4.5 cm yr^{-1} (ref. 6). We have some 40 refraction lines each about 80 km long

covering the area on an orthogonal grid. Most data were obtained from free-floating buoys and some from ocean bottom seismometers, using standard seismic refraction techniques. The upper mantle velocities obtained from these data, which show strong anisotropy, are discussed by Snijders *et al.*⁷.

Using only the first arrivals, the mantle intercept times and crustal velocities were measured and then averaged in age intervals after correcting for the water column travel time.

Fig. 4 Graph for line 8 showing trade-off between LVZ parameters and thickness of the overlying layer, found using the PmP critical point.

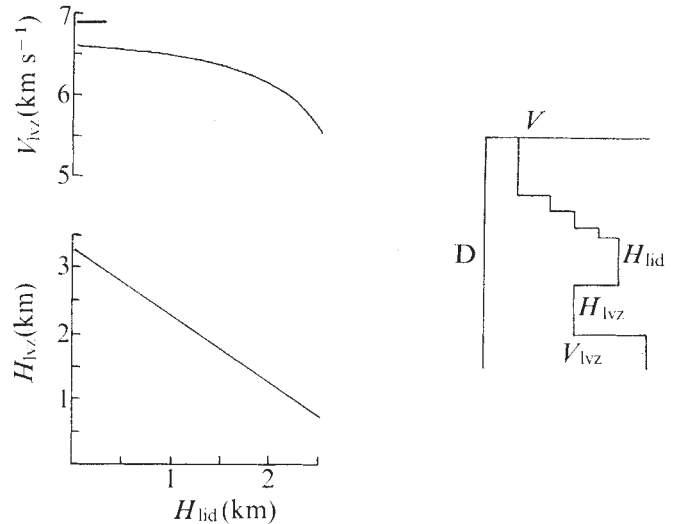
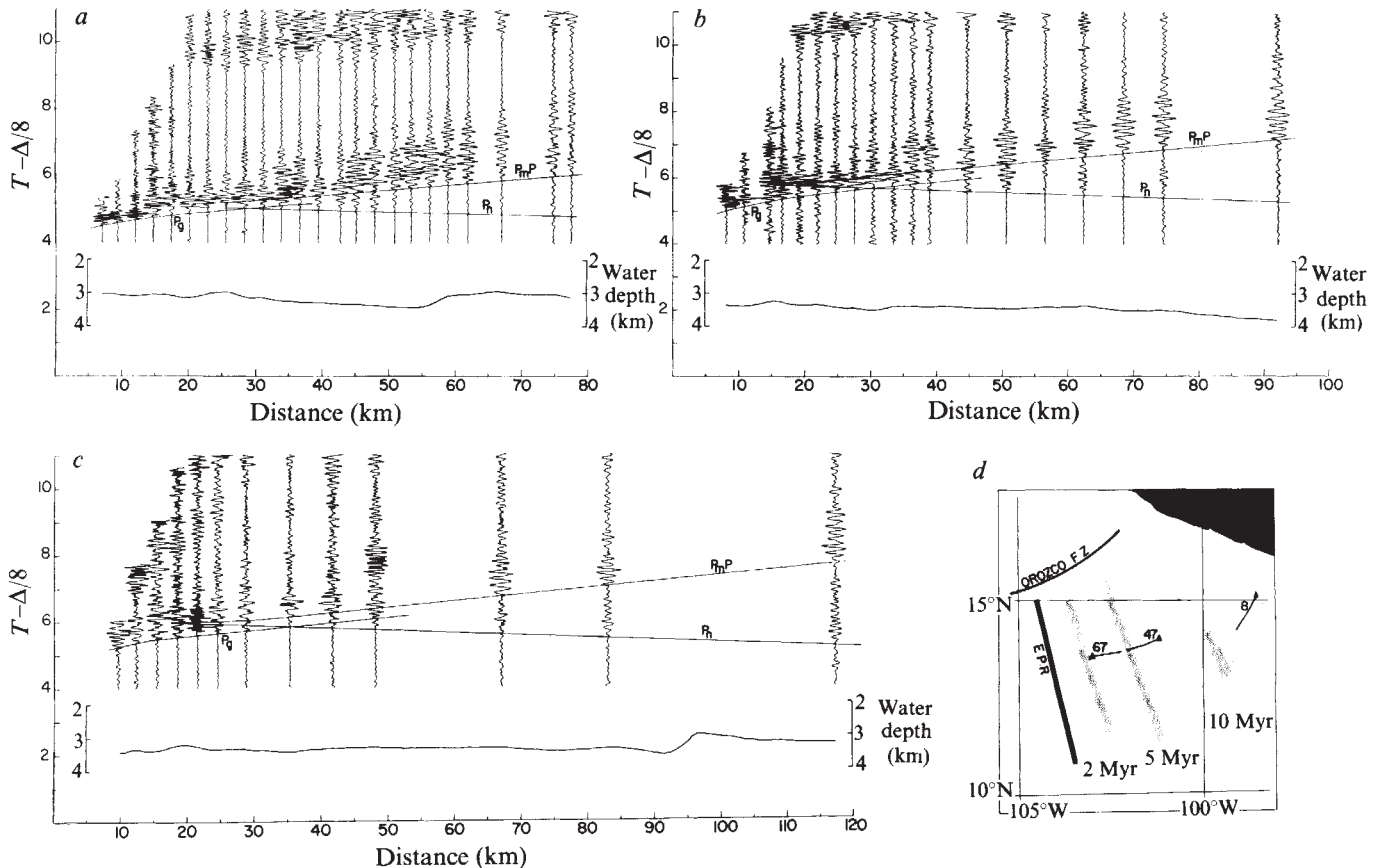


Fig. 3 Record sections from seismic refraction data taken at: *a*, 165; *b*, 275, and *c*, 600 km from the rise crest (lines 67, 47 and 8). The data are obtained by firing a sequence of explosive shots along a line from an array of telemetering buoys. The seismograms are then plotted on a reduced time scale, defined as $T_{\text{reduced}} = T_{\text{observed}} - \text{distance}/8.0$. On this scale horizontal lines correspond to 8.0 km s^{-1} . The travel time curves on each record section are interpretations based on correlating arrivals from one record to another. *d*, Base chart of area studied.



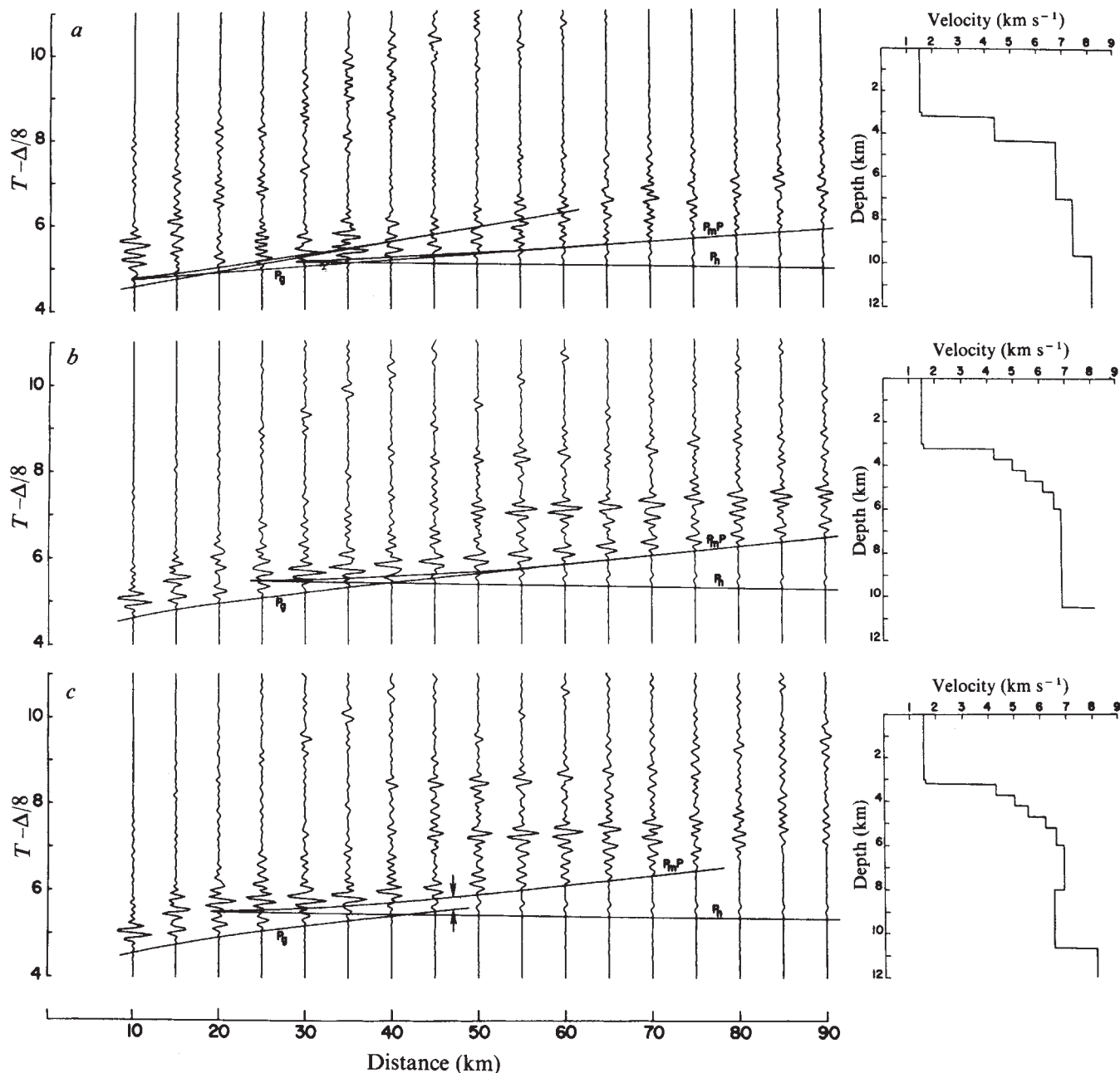
These averages were then plotted against (age)^{1/2} (Fig. 1). The (age)^{1/2} abscissa was chosen since the most rapid changes due to cooling can be expected in the early history of the crust⁸. From Fig. 1a it is clear that the averaged intercept time increases with age by about 0.5 s in 12 Myr. Of this increase only about 0.1 s could be associated with an increasing upper mantle velocity. We therefore conclude that the crust is thickening with age.

In obtaining the upper crustal structure we have used both the travel times and the apparent velocities measured across the 3.0-km long array of five receiving buoys. An example of the apparent velocities we obtained is provided by the data from line 67 (Fig. 2). This line was notable for the small drift of the buoys (< 1 km in 4 h) and the smooth topography. The travel times for this line are shown by the unfiltered record section in Fig. 3. In deriving a model for the lines, it was found that the simplest model which fits both the travel time and apparent velocity data within the observational error was a

roughly linear velocity increase in the upper crust followed by a decrease in the gradient with depth. Theoretical apparent velocities from such a model are shown in Fig. 2 superimposed on the data. Although there is some smoothing of the apparent velocities introduced by the finite length of the array the existence of a discrete velocity jump associated with the layer 2-layer 3 boundary clearly is not supported, since this would appear as a discontinuity in the apparent velocity curve near 15 km. This conclusion is supported by all the lines for which we have good array velocity data and also by the results of Orcutt *et al.*¹⁰. The layered velocity model of the crust, usually assumed, seems to be a simplification and approximation but not a realistic physical model in this area.

To examine the crustal thickening with age, record sections were constructed of selected lines and velocity models which fit both first arrivals and later arrivals were constructed using ray tracing methods, synthetic seismograms and critical point locations. Three such lines (oriented perpendicular to the

Fig. 5 A comparison of synthetic seismograms computed for three types of models. Model *a* has a basal high-speed layer. Model *b* has an upper crustal structure derived from our travel time and apparent velocity data and a constant velocity lower crust. Model *c* is similar to Model *b* except for the basal LVZ. Note the P_mP-P_g offset and the decrease in the P_mP-P_n critical point distance produced by the LVZ.



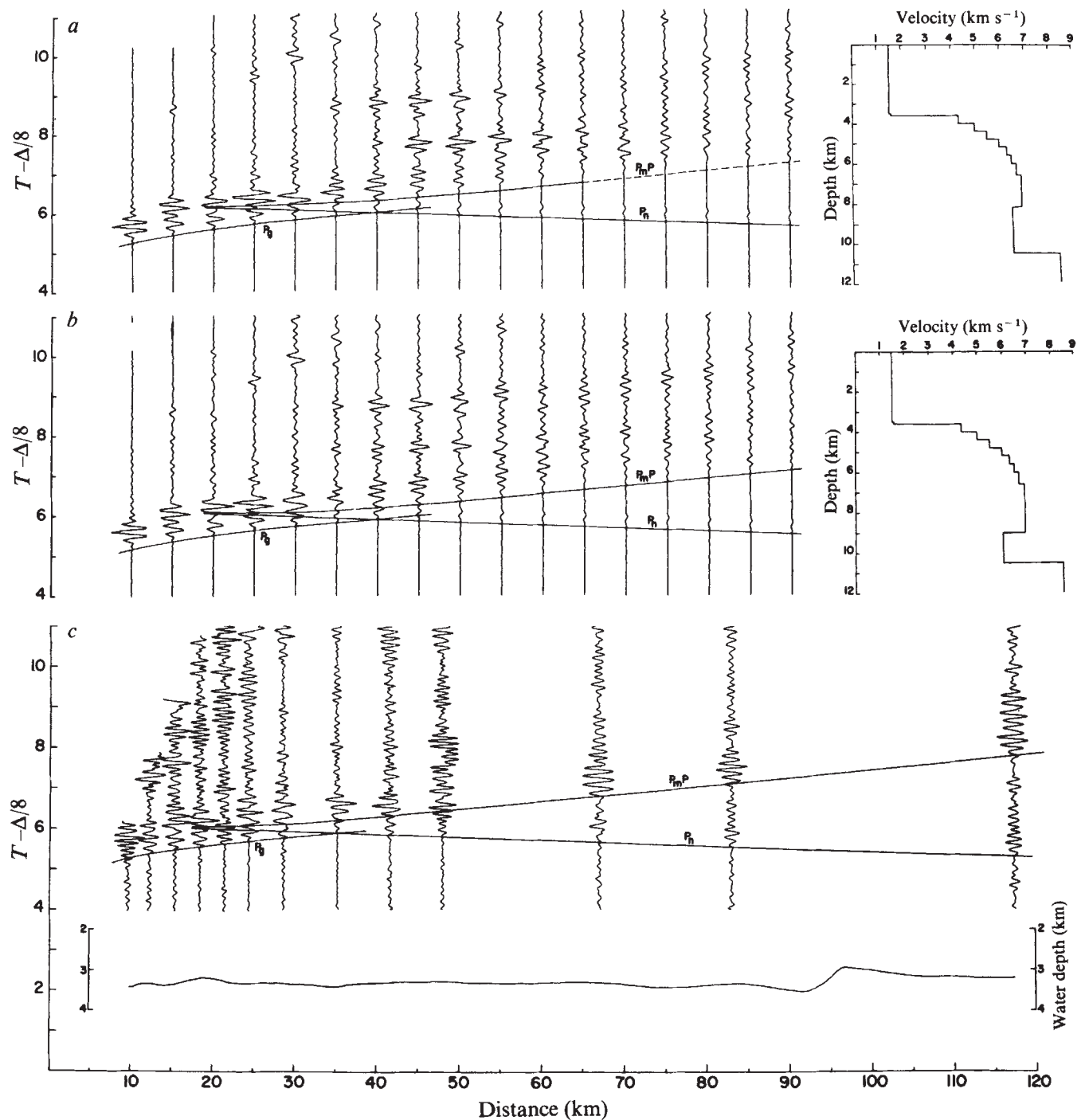


Fig. 6 A comparison of synthetic seismograms computed from the models shown (a, b) with line 8 (c). Model b with the thin LVZ fits the P_mP arrivals at large ranges better than a and is, therefore, the preferred model.

observed magnetic anomalies) are shown in Fig. 3, together with an interpreted travel time curve. Critical to our interpretation are the large amplitude late arrivals at distances greater than 50 km. These arrivals are wide-angle reflections (P_mP) off Moho⁹ and at the largest ranges the apparent velocity, or inverse slope, of these arrivals must be asymptotic to the velocity at the base of the crust or, in the case of a LVZ at the base of the crust, they will be asymptotic to the velocity of the lid overlying the LVZ. One test for the existence of a LVZ is whether these arrivals are tangential to the crustal refracted arrivals or whether they are offset. For line 8 at 600 km from the rise axis there is a clear offset of about 0.35 s. The other two lines also have a well-marked offset of about

0.2 s. This effect is most easily seen by comparing these lines with lines close to the axis (not shown here) which show no offset. The wide-angle reflections also give the velocity of the lid, and in this case, we found velocities of 6.9, 6.8 and 6.9 km s^{-1} respectively.

A second independent test for the existence of a LVZ is the distance and time at which the P_mP critical point occurs on the travel time curves. This, coupled with a knowledge of the upper crustal structure and P_n velocity, can be used to determine if a LVZ exists at the base of the crust. For the lines shown in Fig. 3 the P_mP critical point is fairly clear and we can determine the maximum range at which the critical point may occur. Using the measured critical points, the derived upper

crustal structure, and the asymptotic velocity of P_mP , we can determine if a LVZ is required at the base of the crust.

Using this method, we find that our three lines do require a LVZ at the base of the crust. There is, however, a trade-off between the thickness and velocity of the LVZ and the thickness of the lid, as shown in Fig. 4 for line 8. A further constraint may be placed on the LVZ by the distance to which energy in the P_mP path is propagated since the thickness of the layer overlying the LVZ strongly controls this feature (that is, a thick lid will propagate energy further than a thin lid).

To test our model, further, we synthesised seismograms, using the method of Fuchs and Muller¹¹ as modified by Kennett¹² for marine situations, for three different types of models. These models labelled *a*, *b*, and *c* in Fig. 5 include a model with a high-speed layer at the base of the crust (model *a*), a model with an upper crustal structure similar to that found for line 67 with no basal LVZ (model *b*), and a model similar to *b* but with a basal LVZ (model *c*). Model *a* clearly does not fit our data since it does not produce the high amplitude arrivals in the 15–20-km range; also, the slope of the P_mP arrivals beyond 50 km is 7.5 km s⁻¹ and not the observed 6.8 km s⁻¹. The difference between models *b* and *c* is primarily in the offset between P_mP and P_g and the position of the P_mP – P_n critical point. A comparison of the synthetic seismograms in Fig. 5 and the data in Fig. 3 clearly supports the basal LVZ concept and the criteria for its recognition.

Since the models in Fig. 5 are not specifically related to any line of data, we next tried to compute synthetic seismograms to fit line 8 as accurately as possible. Fig. 6 shows the line 8 record section bandpass filtered from 0 to 8 Hz along with two synthetic record sections computed from models with differently shaped LVZs. Both models give a close fit to the P_g and P_n arrivals; however, the model with a thin LVZ and a higher velocity contrast fits the observed P_mP amplitudes better at ranges greater than 50 km. Hence the model with the lower velocity in the LVZ seems to fit the data better for line 8; this conclusion was also reached for lines 67 and 47.

These results suggest that the thickening of the oceanic crust as observed here and possibly in other parts of the world is due to the growth of a LVZ at the base of the crust which may be formed by a transformation of upper mantle material of velocity > 8.0 km s⁻¹ into material with velocity < 6.8 km s⁻¹. An inspection of the metamorphic reactions that might operate on a peridotite-type rock in a cooling environment to produce these low velocities suggests only one candidate, a serpentinisation or hydration of the peridotite. The data of Christensen and Salisbury⁴ show that if the upper mantle were composed entirely of olivine then a 50% hydration would be required to lower the velocity from 8.43 to 6.5 km s⁻¹. If this serpentinisation hypothesis is correct, then a method of getting the water to these depths may be provided by the cracking front theory of Lister¹³. In this theory hot rock cooled rapidly by contact with water will crack, facilitating further cooling and cracking. This cracking front may proceed to considerable depths depending in part on the strength of the rock. Lister¹³, in discussing this problem, had suggested such a mechanism for the formation of the Moho boundary.

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J. Tuzo Wilson Knolls: Canadian hotspot

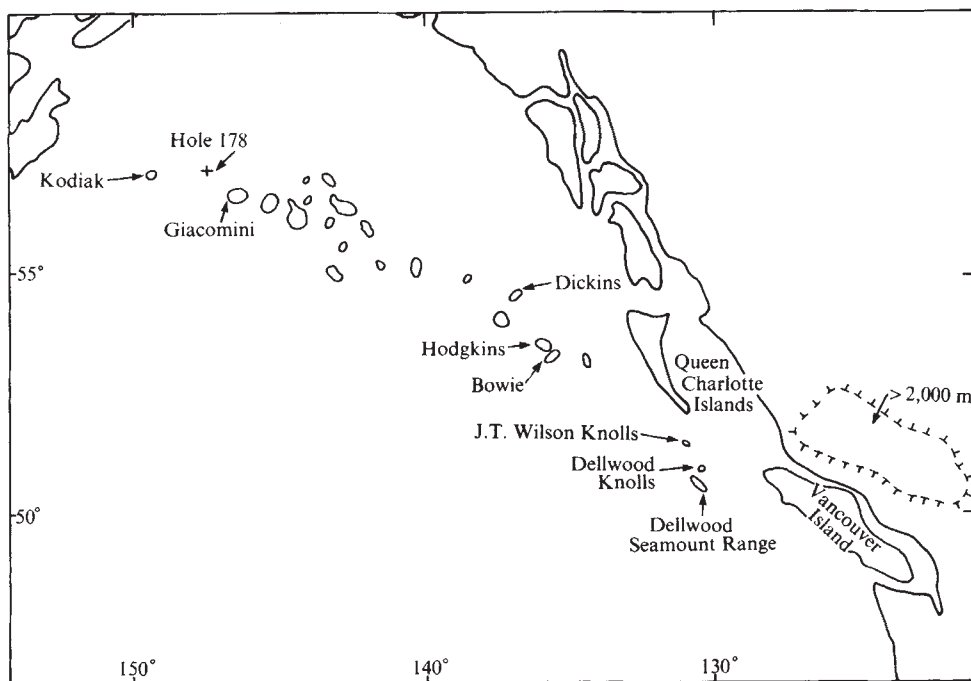
A YOUNG submarine volcano at the base of the continental slope west of Canada has been named after the Canadian geophysicist J. Tuzo Wilson. The volcano is capped by hawaiite, and has an angular separation from a proposed pole of rotation of the Pacific plate and the hotspot frame of reference close to separations for the Kodiak–Bowie seamount chain, suggesting that a hotspot which generated the seamount chain now lies beneath the young volcano. Radiogenic ages of some seamounts of the chain support this hypothesis.

The generation of a line of seamounts (Fig. 1), trending south-east from Kodiak to Bowie Seamounts in the Gulf of Alaska, has been ascribed to a hotspot beneath the Pacific Plate^{1,2}. Bathymetric, geometric, chronological and petrological evidence presented here suggests that the hotspot is now located at the foot of the continental slope, off Queen Charlotte Sound, British Columbia.

In 1973, fresh glassy volcanic rock was raised in two dredge-hauls made on J. Tuzo Wilson Knolls (JTW) at latitude 51° 23'N, longitude 131°02'W and latitude 51°25'N, longitude 131°01'W, respectively (Figs 2 and 3). The knolls which rise from the base of the continental slope 60 km south of Cape St James, Queen Charlotte Islands, lie in an area of high sedimentation rate. The dredged rock shows little sign of manganese encrustation or alteration, and most likely was erupted recently, probably since the diminution of rate of sedimentation following the rise in sea level at the end of the last glaciation. The knolls lie at the southern end of Queen Charlotte fault, a transform fault which forms the boundary between North American and Pacific lithospheric plates^{3–5} and lies north of the triple junction of the North American (NOAM), Pacific (PCFC) and Juan de Fuca (JDFA) lithospheric plates⁵. Oshawa Rise, a broad basement swell, connects the region of the knolls to Bowie Seamount, at the south-eastern end of Kodiak–Bowie Chain^{1,4}. Minster *et al.*⁶, in a global synthesis of movements of lithospheric plates relative to the hotspot frame of reference (HSPT), computed that PCFC–HSPT rotation during the past 10 Myr has been 0.83° Myr⁻¹ about a pole at 67.3°N, 120.6°W. Colatitudes of seamounts of the Kodiak–Bowie Chain relative to this pole (Table 1) are in the range 36.9°–39.4°. The colatitude of JTW is 37.1°. After calculating the longitudinal difference between JTW and a seamount of the chain about the PCFC–HSPT pole, and assuming the rate of rotation of 0.83° Myr⁻¹, one may calculate the age difference between the seamount and JTW (Table 1, rows 4 and 5). Radiogenic ages of Giacomini and Kodiak seamounts, at the northern end of the chain (Table 1, rows 6 and 7) compare closely with calculated seamount–JTW age differences, as does the radiogenic age of basalt from nearby DSDP hole 178. Radiogenic ages of Bowie, Hodgkins and Dickens seamounts, near the southern end of the chain, are 5–6 Myr younger than the calculated age differences. Assuming that volcanism, once initiated at a seamount, has a duration of several Myr, such age discrepancies are not incompatible with the hypothesis that the active end of Kodiak–Bowie Seamount Chain lies at or near JTW, and that the rotation PCFC–HSPT has been constant for the past 22 Myr. Alternatively, the discrepancies may indicate variation in PCFC–HSPT motion through time.

Other possible locations for the active end of the Kodiak–Bowie chain on the Pacific plate are Dellwood Knolls and the south-eastern end of Dellwood Seamount Range (Figs 1 and 2), relevant colatitudes of which are 37.4° and 37.8°, respectively. The south-eastern end of Dellwood Seamount Range lies at the

Fig. 1 Location of Kodiak-Bowie Seamount Chain in Gulf of Alaska. Ticked dashes enclose area of Coastal Mountains of southwestern British Columbia characterised by elevations > 2000 m.



northern end of Explorer Ridge. Dellwood Knolls lies to the east, on the eastern side of Revere-Dellwood fracture zone^{5,7} (Fig. 2). Basalt without significant coating of manganese-iron oxides has been recovered at both of these sites. Analyses of major elements and norms of volcanic rock from JTW, Dellwood Knolls and Dellwood Seamount Range (Table 2) reveal that the latter two features are composed of low potassium tholeiite characteristic of spreading ridges, whereas JTW is composed of much more alkaline hawaiites, characteristic of the terminal phases of seamount volcanism. Thus petrology indicates that JTW is the most likely of the three features to be generated by a hotspot.

If initiation of volcanism at JTW occurred several Myr ago, the generating hotspot would now lie east of the plate margin under the North American plate. If this is true, geometry

dictates that the hotspot lies on the same small circle about the PCFC-HSPT pole as the Kodiak-Bowie chain. A broad positive topographic anomaly and alkaline volcanicity should characterise such a hotspot⁸. In the coastal mountains of British Columbia, a topographic high does occur, centred at 51°N, 125°W (Fig. 1), but the colatitude (PCFC-HSPT) of this area is only 35°, and no young alkaline volcanicity is known from it.

Thus the tentative conclusion is that the Kodiak-Bowie Hotspot is at present located near J. T. Wilson Knolls.

The decision to name this topographic feature after the Canadian geophysicist J. Tuzo Wilson stems principally from its connection with an underlying hotspot. Professor Wilson was the originator of the hotspot hypothesis, and it is thus fitting that a hotspot be named after him, particularly one

Fig. 2 Relation of J. Tuzo Wilson Knoll to neighbouring features. Queen Charlotte Fault follows continental margin off Queen Charlotte Islands. Heavy straight line indicates locations of continuous seismic reflection profile (Fig. 2). Triangles indicate sites of dredged rock for which analyses are listed in Table 2. Contours in m.

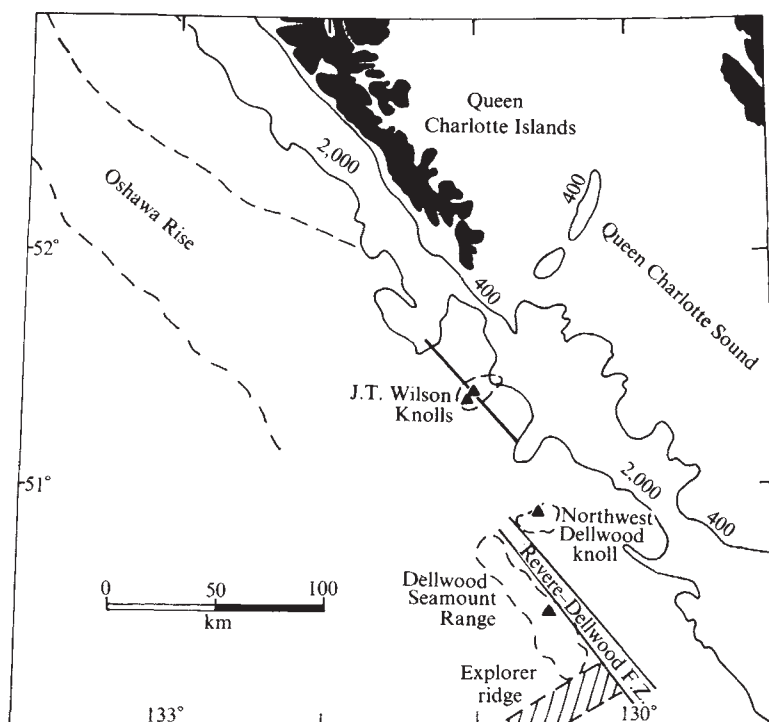


Table 1. Position and age data for Seamounts studied

	J. T. Wilson	Bowie	Hodgkins	Dickens	Giacomini	DSDP 178	Kodiak
Co-latitude about PCFC-mantle Pole (deg)	37.1	37.4	37.4	36.9	38.8	38.6	39.4
Angular distance from JTW about PCFC-mantle Pole(deg)	0	5.5	6.1	7.8	16.6	17.6	18.9
Calculated age (Myr)	0	6.7	7.4	9.4	20.0	21.2	22.8
K/Ar age (Myr)	—	0.1±0.1	2.65±0.2	3.7±0.2	19.9±1.0	22-23	22.6±1.1
Fission-track age (Myr)	—	—	—	—	19.3±3.8	—	25.3±4.3
Age of magnetic anomaly (Myr)	<10	18	19	20	46	47	50
Age-difference (Myr) magnetic-calculated	—	11	12	11	26	26	27

close to Canada. He introduced the concept of transform faults and, with Frederick Vine, first delineated plate boundaries in the north-eastern Pacific. Thus it is fitting that his hotspot lie in the north-eastern Pacific, near the southern end of the Queen Charlotte transform fault.

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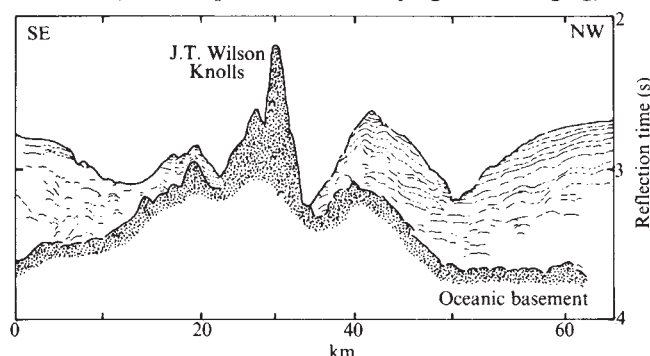


Fig. 3 Continuous seismic reflection profile across J. Tuzo Wilson Knoll. Sedimentary strata cut by canyons of the continental slope cover flanks of volcanic edifices rising from oceanic basement. Vertical exaggeration is 21:1.

fessor R. T. Prider and Dr R. Binns, for providing analytical facilities at the University of Western Australia, Dr Stirling Shaw, Macquarie University, for X-ray diffraction analysis of rock samples, the Institute of Oceanographic Sciences (Wormley, UK) for office and library facilities, Mrs Angela Watters and Ms K. Hayne, for assistance in preparation of the manuscript. This work was supported by grants from the National Research Council of Canada and the Defense Research Board of Canada. The knoll was discovered during a joint cruise of the Institute of

Cerium in chert as an indication of marine environment of its formation

THERE are few if any investigations of deep-sea cherts or siliceous sediments from the geochemical point of view, although several geological and mineralogical studies have been published¹⁻⁶. We present here data on rare-earth elements (REE) in deep-sea cherts, and compare them with relevant data on seawaters as well as cherts exposed on land.

Behaviour of cerium differing from that of other REE in marine environments is already known⁷⁻¹⁰ with particular reference to manganese geochemistry, but a similar distinction in predominantly siliceous sedimentary rocks has not been reported, to our knowledge.

REE in samples were determined with stable isotope dilution technique with relative uncertainties of <1.5%. Our investigations were performed on four deep-sea cherty rocks recovered during the Deep Sea Drilling Project, two microfossil separates, and three terrestrially exposed cherts, including an Archaean one from Gunflint, Canada. The microfossils were separated from radiolarian ooze by the sedimentation technique. The microfossils under consideration are siliceous and substantially free from calcareous ones¹¹. Excepting 4-29C-1 (chert from the Caribbean Sea), the other deep-sea samples are from the central Pacific.

The results of REE determination are given in Table 1. Figure 1 shows the chondrite-normalised REE patterns for deep-sea samples; the REE abundances in the Leedey chondrite¹² are employed for the Masuda-Coryell plotting (plotting the logarithm of chondrite-normalised value against the order of atomic number of REE). It should be noted that all of the DSDP samples investigated show large negative Ce anomalies. The 'Ce anomaly' here is defined as the ratio of observed Ce abundance to that which would fall on the La-Nd join in the Masuda-Coryell plot. The Ce anomaly ranges approximately from 0.2 to 0.3, with the exception of the Caribbean chert (0.467).

The absolute concentration levels of REE in the four highly siliceous sediments show a large variation decreasing in the order 7-66-5, 16-163-14, 4-29C-1 and 17-167-85. Sample 7-66-5 is cristobalitic porcellanite, the next two samples are cristobalite cherts, and the last one, 17-167-85, is a quartz chert. Thus, the

Table 2. Analyses of major elements and norms of volcanic rocks

	Hawaiite J. T. Wilson Knolls	Hawaiite J. T. Wilson Knolls	Qz-tholeiite Dellwood Smt Ra.	Ol-tholeiite Dellwood Knolls
SiO ₂	49.47	51.47	50.38	47.48
TiO ₂	2.24	1.51	1.48	1.16
Al ₂ O ₃	17.25	17.83	14.71	15.87
Fe ₂ O ₃	1.67	1.23	1.80	1.29
FeO	6.75	5.17	7.38	7.69
MnO	0.14	0.13	0.18	0.16
MgO	4.52	5.06	7.12	10.71
CaO	7.95	8.16	12.53	11.26
Na ₂ O	4.94	4.74	2.51	2.21
K ₂ O	2.47	2.26	0.26	0.18
H ₂ O ⁺	1.36	1.56	1.95	0.64
H ₂ O ⁻	0.45	0.33	0.03	0.09
P ₂ O ₅	0.92	0.77	0.13	0.09
	100.13	100.22	100.46	98.83
Q	—	—	0.33	—
Or	14.60	13.35	1.54	1.06
Ab	27.53	32.12	21.24	18.70
An	17.60	20.70	28.10	32.85
Ne	7.73	4.33	—	—
Di	12.93	11.94	26.98	18.08
Hy	—	—	14.58	6.82
Ol	9.13	9.45	—	16.30
Mt	2.42	1.78	2.61	1.87
Il	4.25	2.87	2.81	2.20
Ap	2.14	1.79	0.30	0.21

Table 1 REE concentrations (p.p.m.) in cherts and siliceous microfossils, with sample descriptions

	DSDP 7-66-5	DSDP 4-29C-1	DSDP 16-163-14	DSDP 17-167-85	DSDP 7-66-3 microfossil	DSDP 16-162-7 microfossil	750824-14	750824-15	Gunflint
La	36.7	12.35	14.11	3.97	5.67	0.311	4.77	5.15	0.0785
Ce	24.4	12.16	6.62	2.18	2.98	0.1519	10.04	14.94	0.1946
Nd	35.7	12.75	15.62	2.92	6.96	0.355	4.56	5.45	0.0738
Sm	8.03	2.72	3.50	0.531	1.569	0.0797	0.920	1.250	0.01536
Eu	2.08	0.706	0.906	0.1295	0.395	0.01994	0.202	0.271	0.00453
Gd	9.03	3.08	3.75	0.645	1.864	0.0666	0.866	1.115	0.01790
Dy	9.21	2.87	3.81	0.598	1.835	0.0802	0.727	1.024	0.0222
Er	5.62	1.674	2.27	0.388	1.157	0.0458	0.451	0.544	0.01681
Yb	—	1.440	2.03	0.355	1.059	—	0.445	0.492	0.01632
Lu	0.842	0.219	0.316	0.0551	0.1893	0.00669	0.0672	0.0731	0.00236
Ce anomaly*	0.321	0.467	0.217	0.290	0.235	0.224	1.02	1.37	1.21
Latitude	2°23'N	14°47'N	11°15'N	7°4'N	2°23'N	14°52'N	Kamiasô		Sibley
Longitude	166° 7'W	69°19'W	150°18'W	176°50'W	166° 7'W	140° 3'W	Gifu, Japan		Ontario,
Water depth(m)	5293	4247	5320	3176	5293	4854			Canada
	Oligocene– Upper Cretaceous	Middle Eocene	Middle Eocene– Upper Cretaceous	Lower Cretaceous	Oligocene	Middle Eocene	Permian–Jurassic		2.0–2.4 ×10 ⁹ yr
Siliceous microfossils	rare	common	none	none	abundant	abundant	abundant	abundant	none
Lithology	cristobolitic porcelanite	chert	chert	chert	radiolarian ooze	radiolarian ooze	chert	chert	chert
SiO ₂ mineral	cristobalite (opal-CT†)	cristobalite (opal-CT†)	cristobalite (opal-CT†)	quartz			quartz	quartz	quartz

*See text.

†After the opal classification by Jones and Segnit¹⁹.

absolute REE abundance levels in these cherty rocks seem to depend on the degree of diagenesis. With the present limited data, it is hard to discriminate on relative REE patterns between cherty rocks and microfossil separates. In general, La, Nd and Sm fall on a straight line, while Sm, Gd and Dy fall on another straight line; there is a small break at Sm.

Figure 2 shows the chondrite-normalised REE patterns of four terrestrially exposed cherts; one is a Transvaal chert analysed by Wildeman and Haskin¹³ for REE. We have studied two cherts from Kamiasô, Gifu, central Japan, (Triassic–Jurassic) and a Gunflint chert (2.0–2.2, or 2.43 × 10⁹ yr) (ref. 14), Canada. REE contents in the Gunflint chert are similar to but somewhat lower than in the Transvaal sample (about 2.0 × 10⁹ yr) (ref. 13). A remarkable observation from Fig. 2 is that these cherts exposed on land either show a small positive Ce anomaly or seem to have no Ce anomaly at all, in contrast to deep-sea cherts. Kolodny and Epstein¹⁵ have revealed a clear distinction in $\delta^{18}\text{O}$ between terrestrially exposed and deep-sea cherts. They conclude that the lower values of $\delta^{18}\text{O}$ of terrestrial cherts compared with DSDP cherts are attributable to the shallower water environment in which the terrestrial cherts were formed. This conclusion is consistent with that derived from the present study.

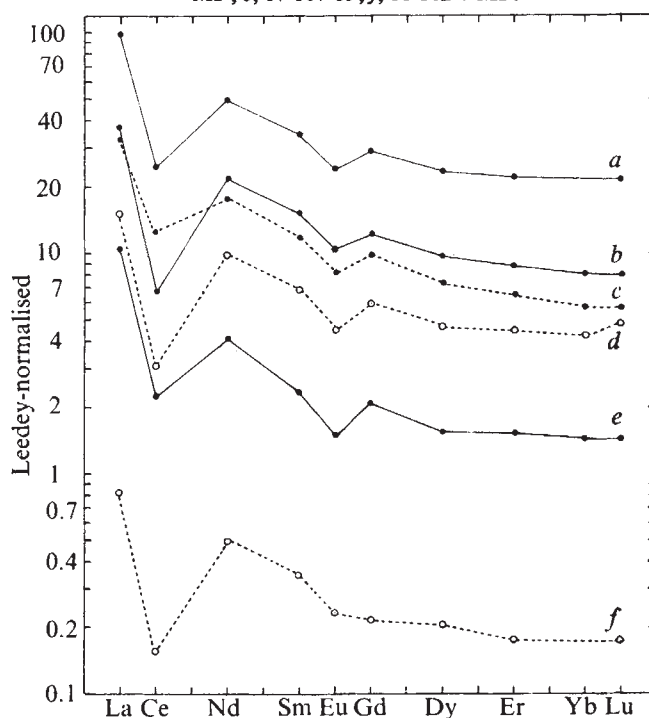
Goldberg *et al.*² found that Ce in a seawater sample was depleted by a factor of 0.23 relative to the adjacent rare-earth elements. The corresponding Ce anomaly for eight Atlantic Ocean samples from two stations (45°N, 16°W and 43°N, 28°W) is about 0.32 as a geometrical mean, on the basis of data presented by Høgdahl¹⁶. He also reported, however, that Ce is present in normal concentrations in seawaters of the Puerto Rico Trench, the West Deep Cariaco Trench and Barents Sea¹⁶. It should be noted that these areas are all relatively close to continents and/or the dimensions of the seas in question are small compared with oceans. The water of the Black Sea¹⁷ also does not show a substantial Ce anomaly.

It is evident that the presence or absence of Ce anomaly and its extent and direction of deviation can be a good indicator of aqueous (perhaps marine) environment of formation for cherty rocks. A Caribbean chert indicates a smaller depletion of Ce compared with the Pacific ones. This could be due to the Caribbean Sea being encircled by a chain of islands. The REE patterns of terrestrial cherts can be interpreted as suggesting

that they were formed in coastal areas, marginal seas or land-enclosed seas.

Our results suggest that, in shallow-sea areas, Ce perhaps mostly involved in suspended solid particles behaved apparently in the same fashion as other REE, while in deep-sea areas Ce was subjected to selective removal relative to other REE. Difference in residence time of lithogenous suspended particles is one of the major factors responsible for this areal difference. Comparatively long residence time of suspended particles would promote disintegration into finer particles and their further alteration, which would be in turn favourable with chemical

Fig. 1 Chondrite-normalised REE patterns of deep-sea cherts and siliceous microfossils (MF) separated from DSDP radiolarian ooze. a, sample 7-66-5; b, 16-163-14; c, 4-29C-1; d, 7-66-3 MF; e, 17-167-85; f, 16-162-7 MF.



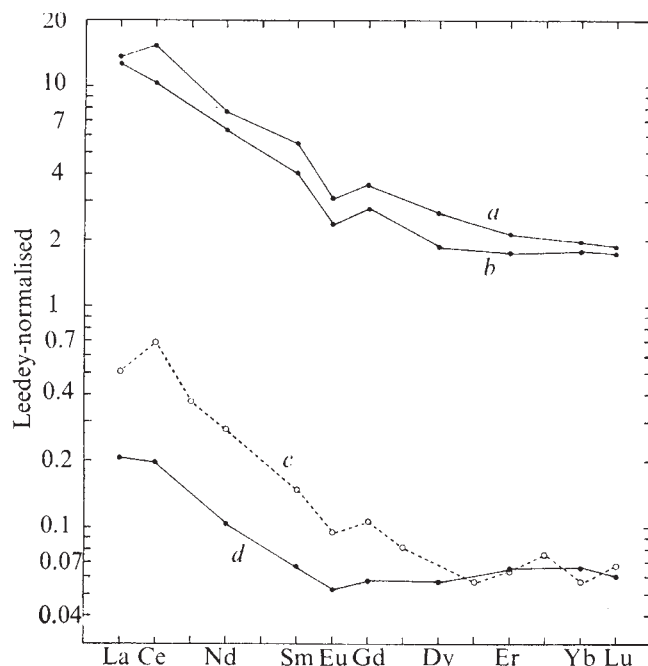


Fig. 2 Chondrite-normalised REE patterns of cherts exposed on land; 750824 samples are from central Japan. *a*, 750824-15; *b*, 750824-14, *c*, Transvaal (Wildeman and Haskin); *d*, Gunflint.

reactions, including oxidation of Ce into the tetravalent form, and separation of Ce from other common REE; without particle disintegration, the size of particles in deep-sea areas can be finer as a result of sorting. This does not necessarily mean that the oxidation into Ce^{4+} starts in deep-sea areas. Høgdahl¹⁸ found no Ce depletion in unfiltered water samples from the River Gironde and its mouth to the sea, while a slight Ce depletion was detected for filtered waters.

Almost all of samples studied display rather small negative Eu anomaly, which is considered to reflect that of a probably continental source material.

Two major theories, biogenic and volcanic, have been advocated to explain the genesis of cherty rocks. If volcanic activity is directly responsible for chert formation, the rocks so formed should not show a negative Ce anomaly. This preliminary investigation suggests that high-accuracy REE determinations can lead to useful results if applied to researches in siliceous sedimentary rocks and related subjects.

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Factors controlling grain weight in wheat

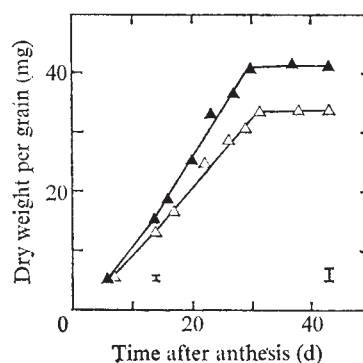
THE physiological factors controlling differences in grain weight between cultivars of wheat are not fully resolved. The experiments described here indicate that final grain weight in two cultivars of wheat is mainly dependent on the rate of accumulation of dry matter, which in turn is governed by the number of endosperm cells formed. Cell number in the endosperm seems to be regulated by the supply of assimilates available to the grain during the first 2 weeks after anthesis.

Two cultivars of wheat (*Triticum aestivum* L.), Maris Huntsman and Val, were grown in pots in the open from November 1975 to July 1976, and tillers were removed to maintain a constant density of 320 shoots per m². The plants reached anthesis on 7 and 8 June, Val being the earlier, and attained a mean grain number per inflorescence of 48.8 (Maris Huntsman) and 48.5 (Val). In the first experiment inflorescences were collected at intervals from anthesis to maturity. Grains were removed from the lower two florets of the 10 central spikelets of the inflorescence, and the weight per grain determined after drying to constant weight. The stage of development of the endosperm was determined from median transverse sections of grain fixed in glutaraldehyde, embedded in epon, and examined from 2-μm sections using phase contrast optics.

At maturity, Maris Huntsman contained 21% more dry matter in the grain than Val (Fig. 1), and the rates of increase in dry weight for the period 14–30 d after anthesis, calculated by regression analysis, were 1.63 ± 0.09 mg d⁻¹ (Maris Huntsman) and 1.12 ± 0.07 mg d⁻¹ (Val). The higher rate in Maris Huntsman could have been due to a greater supply of assimilate, or to its grain being a more efficient sink, or to a combination of the two factors.

It has been suggested¹ that the rate of increase in dry weight of the grain may be closely related to sink size as determined by the number of endosperm cells formed, and our data provide evidence for this. Examination of the developing endosperm showed that by 14 d after anthesis, the outer layer of endosperm cells had ceased to be meristematic in both cultivars, and had differentiated into recognisable aleurone cells, indicating that the number of endosperm cells had been fixed². The effect of cell number in the endosperm on the rate of increase in dry weight and on final grain weight was tested in a second experiment, in which floret number per inflorescence was reduced to four on two dates by removing all florets except the basal floret of the four central spikelets. Florets were removed at

Fig. 1 Dry matter accumulation of the lower two grains of the 10 central spikelets of the inflorescence. Each point is the mean of 64 inflorescences. Vertical bars indicate the least significant differences ($P < 0.05$) at 15 d after anthesis and at maturity. ▲, Maris Huntsman; △, Val.



anthesis to provide an excess of assimilate available to the remaining grains during the period of cell division in the endosperm, and 15 d after anthesis, to give an excess of assimilate during the time when development was due entirely to cell expansion in the endosperm. Inflorescences were collected on three dates between 15 d and maturity, and the weight per grain was determined after drying to constant weight. The number of endosperm cells was determined in grains fixed in 1:3 acetic acid-absolute ethanol and macerated with fungal cellulase³. Grains from the basal floret of the four central spikelets in intact inflorescences were controls.

Reducing floret number at anthesis increased the dry weight per grain at 15 d and the number of endosperm cells in both cultivars, although the effect was larger in Val (Fig. 2, Table 1). Also, the rate of dry matter accumulation

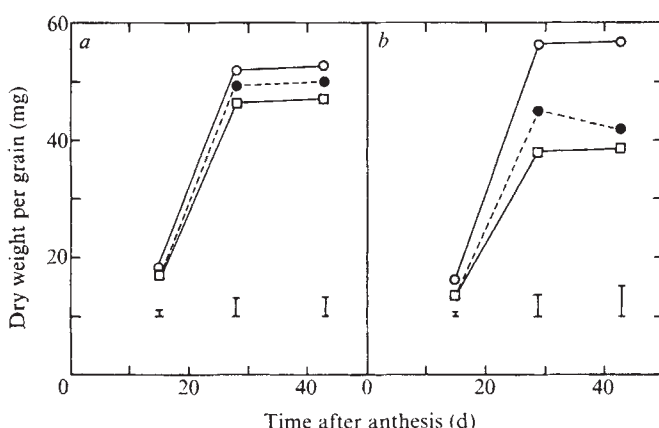


Fig. 2 Dry matter accumulation of the basal grain of the four central spikelets of the inflorescence. *a*, Maris Huntsman; *b*, Val. Each point is the mean of 20 inflorescences. Vertical bars indicate the least significant difference ($P \leq 0.05$) between the control and either treatment. $\square$, Control; $\circ$, florets removed at anthesis; $\bullet$, florets removed 15 d after anthesis.

was stimulated, resulting in an increase in total dry weight at maturity of 13% in Maris Huntsman and 47% in Val. The reduction of floret number at 15 d produced only a small increase in the final dry weight of the grain.

Grain formed in intact inflorescences of Maris Huntsman contained 19% more endosperm cells and accumulated 21% more dry matter at maturity than those of Val. The difference in grain weight between the cultivars was closely correlated with the difference in the number of endosperm cells, suggesting that cell number in the endosperm is a major factor controlling the rate of increase in dry matter and final dry weight. The operation of this control is demonstrated by the small effect of increasing the supply of assimilate to the grain at 15 d, when the number of endosperm cells was fixed and further development was due to cell expansion only.

Grain weight in intact inflorescences seems to be determined mainly by differences in the supply of assimilate to the developing grains, which in turn regulates the size of the grain sink to match the amount of assimilate available.

The implications for plant breeding are twofold. First,

the potential sink size in some wheat cultivars is a function of the number of cells in the endosperm and the level of assimilate supply during the meristematic phase of endosperm development. Second, the number of endosperm cells formed seems to be a reliable indicator for use in selection for the yield component, final grain weight.

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Pattern of lean and fat deposition in adults

THE fact that obesity becomes more common when societies change from a subsistence/labour intensive to an affluent/sedentary way of life might be taken simply as a demonstration that easy living encourages slothfulness and gluttony. Fat people, however, do not in general seem to eat more than thin ones, and there is increasing evidence of an inherited component of obesity. It seems more likely therefore that, throughout much of past history, individuals who had a genetic constitution predisposing them to store energy preferentially as fat have had some kind of advantage for survival. We have used a computer simulation model of energy balance to compare the effects of different seasonal patterns of work output and food availability on individuals with inherent tendencies towards either leanness or fatness, and to illustrate the circumstances and the manner in which the latter type has a relative advantage over the former.

In subsistence agricultural societies, most adults are smaller and take less food than those in affluent communities. The pattern of eating is often feast-and-fast; the heavy manual work of cultivating the next year's crops frequently coincides with reduced availability of food. In these conditions, the ability to store excess energy efficiently and to release it when needed for physical work has survival value. In affluent societies however, a regular, adequate food supply is guaranteed and activity is fairly constant throughout the year. There is not the same need for the body to store and release energy. Rather, people tend to overeat and longevity may be improved by the ability to 'burn-off' excess energy and so avoid obesity with its health penalties. A possible method of meeting these different requirements for optimal survival would be to vary the relative proportions of lean and fat tissue used in storing excess food energy. The efficiency of converting food to fat is about 70% (refs 1, 2); fat tissue has high energy density and low tissue maintenance requirements. Food energy is converted to protein with an efficiency of only 40% (refs 1-3); lean tissue has a low energy density and relatively high maintenance metabolic needs. Both fat and lean tissues are very efficiently mobilised for energy.

We have defined the ratio P as

$$P = \frac{\text{energy stored as (or mobilised from) protein}}{\text{total energy stored (or mobilised)}}$$

Values of P for non-obese subjects can be calculated from the classical starvation studies of Keys *et al.*⁴, and Fig. 1 shows the distribution obtained.

The modal value of P is between 0.05 and 0.10, but a tail extends to much higher values. By contrast, in obese subjects the P values calculated from Passmore's starvation studies⁵ were all very low and ranged from 0.02 to 0.04. In Keys's data there is a significant negative correlation between the value of P and the percentage of the body which was fat, but no correlation between the value of P and the total body weight. The P value is therefore associated with a body type but not a body

Table 1 Effect of reducing floret number at anthesis on the number of endosperm cells formed per grain. Each value is the mean of 10 grains, 15 d after anthesis

	Control	No. of cells Florets removed
Maris Huntsman	145,000	157,000
Val	122,000	158,000
s.e.m.		2,200

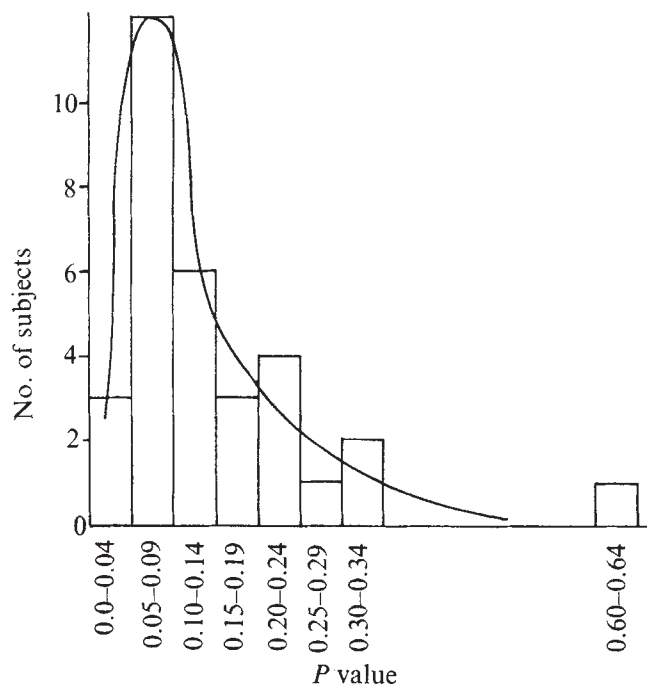


Fig. 1 A histogram showing distribution of values of the ratio of energy mobilised from protein to total energy mobilised. Taken from ref. 4.

size. As the subjects in Keys's experiment were fasted, they lost most of their body fat but there was little change in P value. It seems likely that the value of P is set in an adult and remains fairly constant regardless of present body weight. In children, however, P varies widely with age and there are significant differences between boys and girls during adolescence⁶ (Fig. 2).

The computer model¹⁰ has enabled us to quantitate the effect of different P values among adults in subsistence and affluent societies. For both societies, we have modelled men with constant values of P set at $P = 0.03$ ('fat man') and at $P = 0.30$ ('lean man'). These are near the tenth and ninetieth percentiles of the range of P .

As an example of men in a subsistence community, we have used data from Gambian farmers with food intakes and activities at different times of the year as described by Fox⁷. The model has reproduced the observed weight changes (Table 1).

The total weight changes given by the model were similar in both 'fat' and 'lean' men. The 'fat man' used mainly fat to balance his energy budget, but the 'lean man' drew upon his lean

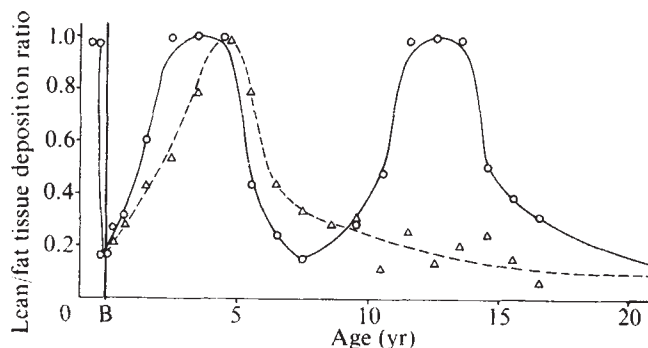


Fig. 2 Variations in children of the ratio calories deposited as protein to total energy deposited. $\circ$, Boys; $\triangle$, girls. Sources as given in ref. 6.

tissue, which is largely muscle, to meet his energy deficits. The 'lean man' therefore depleted his muscles just when they were needed for heavy work. Although no field studies are available to us, it seems reasonable to suppose that the ability of an individual to perform sustained muscular work would be impaired by the loss of muscle tissue. In acute experiments⁴ loss of handgrip power is correlated with loss of body lean tissue. The utilisation of fat as a storage medium therefore has two advantages; it is not only more efficient but also spares muscle tissue which is needed for manual work.

In affluent societies, body weight is slightly higher and maintenance energy needs are also slightly higher in consequence. In contrast to the Gambians, there are only minor seasonal variations in food intake and activity, and there is a tendency for intake to rise above maintenance needs. We have modelled the effects in the 'fat man' and the 'lean man' of 5% and 10% increases of intake above the requirement for weight stability. The mean food intake was kept constant at this new level and there was a 20% random variability about the mean intake to simulate daily variability⁸ (Fig. 3).

The model predicts that body weight would rise and take 3–6 yr to reach new equilibrium levels. Table 2 shows that with a constant 10% excess of energy, the 'fat man' gained 18.1 kg in body weight, but the 'lean man' only 8 kg. More significant are the changes in body composition. In the 'fat man' the per cent body fat rose from 12% to 23%; the 'lean man' rose from 12% to 13%. Since the ill effects of obesity are probably related both to excess weight and excess fat, the 'fat man' may well suffer a double penalty for overeating the equivalent of one small chocolate bar a day.

In contrast to adults, the P values in children change during growth (Fig. 2). Using a modification of our computer model,

Table 1 Seasonal patterns of weight change and body composition for subsistence farmers, derived from computer model

	Total body weight, W (kg)	ΔW	Body fat, F (kg)	ΔF	Body lean L (kg)	ΔL	% Fat
'Fat man'							
Starting weight (59)	59.0	-4.0	11.3	-3.4	47.7	-0.6	14
5 months cultivation food $\uparrow$ work $\uparrow$ (55)	55.0		7.9		47.1		10
4 months harvest food $\uparrow$ work $\downarrow$ (60)	60.7	+5.7	12.7	+4.8	48.0	+0.9	15
3 months rest food $\downarrow$ work $\downarrow$ (59)	59.8	-0.9	11.9	-0.8	47.9	-0.1	14
'Lean man'							
Starting weight	59.0	-3.1	11.3	-0.9	47.2	-2.2	14
5 months cultivation food $\downarrow$ work $\uparrow$	55.9		10.4		45.5		13
4 months harvest food $\uparrow$ work $\uparrow$	60.7	+4.8	11.9	+1.5	48.8	+3.3	14
3 months rest food $\downarrow$ work $\downarrow$	59.4	-1.3	11.4	-0.5	48.0	-0.8	14

Observed weights⁷ in parentheses.

we have shown⁶ that a sustained shift up or down in the pattern of P values throughout childhood would produce substantial changes in body composition, equivalent to ectomorphy and endomorphy, even though food intakes were unchanged.

In adults it seems that the P value has implications for body composition, and in some circumstances, for body weight also. In subsistence communities it seems advantageous to use fat as the main energy store, and thus to spare muscle in conditions of stress. The low P value associated with this pattern of adaptation seldom produces obesity in these conditions because of the limited availability of food. As communities become more affluent, however, the survival advantages of a low P value no longer apply, as obesity and its complications become an increasing problem for the men with the low P values. The

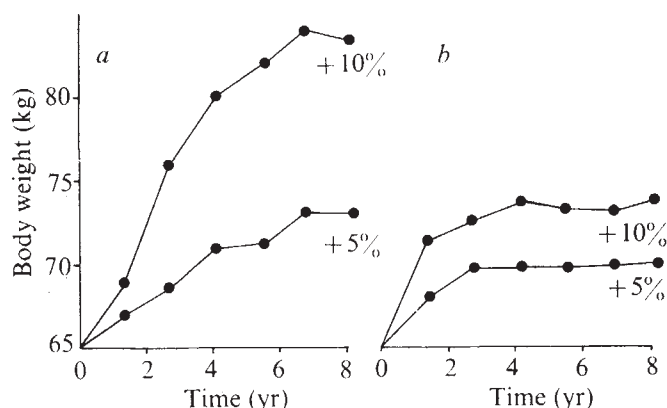


Fig. 3 Changes in body weight associated 5% and 10% above maintenance food intake. *a*, $P = 0.03$; *b*, $P = 0.30$.

shape of the curve in Fig. 1 is compatible with a genetic basis for the distribution of P values. These data are derived from North American men, but have a low modal value, that is a 'fat man' value, consistent with adaptation to subsistence conditions. The presence of some individuals with a high P value might indicate genetic drift. Such a genetic mechanism could explain the inherited pattern of obesity commonly found in affluent countries. In affluent conditions, a genetic drift of the P value to higher levels would have no survival disadvantages and might even have positive advantages although these would occur mainly after reproductive age. If the affluent society persists, there may thus eventually be a higher proportion of 'lean' individuals.

James and Trayhurn⁹ have speculated on the enzyme pathways which might be responsible for the utilisation of fat and protein to meet energy needs and we are developing a more sophisticated computer model to try to localise the critical pathways. The apparent importance of the P value in determining human body composition suggests that treatment of obesity could be effected by chemotherapy to raise the value of P as an alternative to present methods which stimulate the basal metabolic rate or depress appetite. If the P value could be raised

from 0.03 to 0.30, then our model suggests that an obese man would slowly lose weight and fat without altering his energy intake.

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Dominance and reproductive success among female gelada baboons

THE relationship between dominance and reproductive success has been of considerable theoretical interest, with an extensive literature reporting its existence among males in many species¹. Although female dominance hierarchies are known to exist in various species, the possibility of a similar relationship for females has not been investigated in detail. Indeed it is often assumed that Bateman's principle² implies that intrasexual selection (and hence the effectiveness of dominance as a reproductive strategy) will be very much reduced among females. Only one study of mammals presents unequivocal evidence of a relationship between dominance and reproductive success for females³, although there is indirect evidence that it may occur in several other species⁴⁻⁷. We report here evidence for such a relationship in the case of female gelada baboons (*Theropithecus gelada*) in the wild.

Gelada baboons live in reproductive units that typically consist of a single adult male and up to 10 mature females with their offspring⁸. During a field study of this species in Ethiopia in 1974-5, dominance ranks among the females in each of 11 reproductive units were determined by analysing the frequencies of approach-withdrawal type agonistic interactions within each unit. In all cases, the hierarchies were found to be strictly linear.

The number of immature animals less than 3.5 yr old at the start of the study was known for each unit, together with the number of infants born into each unit during the subsequent 9 months of intensive study. From detailed studies of the social structure of these units, it was possible to determine probable mother-offspring relationships with some confidence. Survivorship for immature animals up to the age of 3.5 yr was estimated to be 80% (unpublished data). Thus, on average, the data for each unit will deviate from either the true number of births or the number of offspring surviving to the age of 3.5 yr by only about 10%. In view of this, no attempt was made to correct the raw data in any way.

Figure 1 shows the mean number of offspring per female for females of the various dominance ranks. There is a significant correlation between these two variables ($r_s = 0.667$, $n = 8$, $P < 0.05$), and a linear function has been fitted to the data by the method of least squares. In practice, a linear function is *a priori* unlikely, because, no matter how low a female's dominance rank may be, she will have some finite (albeit small) probability of reproducing. But, fitted exponential and hyperbolic functions give only marginal increases in goodness of fit.

Table 2 Computer model predictions of changes in body weight and composition with overfeeding of 'fat' and 'lean' men

	Total weight, W (kg)	ΔW	Fat tissue, F (kg)	ΔF	Lean tissue, L (kg)	ΔL
Starting values	65		11		54	12
Final levels with food intake 5% above maintenance requirements						
'Fat man'	73	+8.0	17.7	+6.7	55.3	+1.3
'Lean man'	68.9	+3.9	12.0	+1.0	56.9	+2.9
Final levels with food intake 10% above maintenance requirements						
'Fat man'	83.1	+18.1	26.2	+15.2	56.9	+2.9
'Lean man'	73.0	+8.0	13.1	+2.1	59.9	+5.9

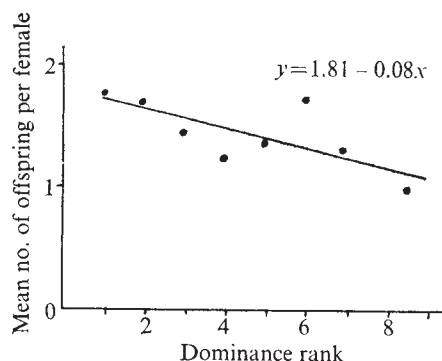


Fig. 1 Relationship between the mean number of offspring per female and rank in the female dominance hierarchy (see text for details).

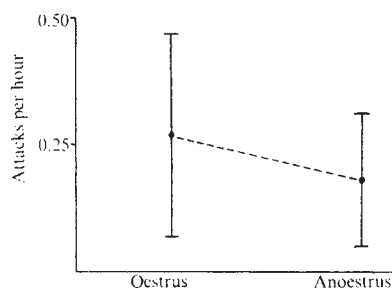
Since the linear function does not yield a zero value for reproductive output until the equivalent of rank 23 (a value well beyond the normal range of unit size for this species*), a linear approximation is quite adequate for most purposes.

Two alternative explanations for these results might lie in a confounding of variables. First, it could be argued that, as the number of females in a unit increases, mutual interference among them might result in a lowering of each of their individual reproductive rates by the same amount. The fact that the samples for low ranks are based on large units, whereas those for high ranks are based on both small and large units, will tend to bias the results in favour of the latter. Consequently, the average output would seem to be higher for dominant females than for subordinates, although there was no underlying relationship between dominance and reproductive success. That this is not so in the present case can be shown by comparing the reproductive outputs of the top three ranking females in small units (four or five females) with those of the top three females in large units (six to nine females). The former averaged 1.889 offspring per female, compared with 1.778 for the latter: the difference is not significant (randomisation test: $t=0.509$, d.f.=16, $P>0.0$). Second, it could be argued that, because the number of offspring a female can expect to have will be in part a function of her age, the results obtained could be due to a relationship between age and dominance rank. It can be shown, however, that there is no significant relationship between these two variables; furthermore, it can also be shown that, while there is a relationship between age and number of offspring, this is independent of the relationship between dominance and number of offspring (unpublished data).

The available evidence suggests that dominant females achieve this reproductive differential by harassing subordinate females, thereby causing them to undergo more oestrous cycles before conception than do dominants. This can be seen from two relevant sets of data.

First, Fig. 2 compares the frequencies with which a sample of nine females were harassed by females in their units who were more dominant to them when they were in oestrus and when they were out of oestrus. For these purposes, oestrus is defined

Fig. 2 Mean frequency of attacks received from the more dominant females of their units by females who were in the oestrous and anoestrous parts of the menstrual cycle (vertical bar gives 1 s.d. either side of the mean).



as the part of the menstrual cycle during which the areas of sexual skin are surrounded by fluid-filled vesicles, sexual activity being most common during this period^{9,10}. The difference between the two conditions is significant (randomisation test for matched pairs: $P=0.023$, $n=9$), attacks being about 50% more common during oestrus than during anoestrus.

Second, an examination of (1) the order in which sets of females which were synchronised in their menstrual cycles stopped cycling and (2) the order in which sets of females who gave birth within a period of 2 months of each other did so indicates that the order of (probable) conception corresponds closely to the order of dominance ranks. To maintain statistical independence, the data were analysed by considering only successive dyads in each set: in other words, triads, for example, were treated as two sets of successive dyads, *A-with-B* and *B-with-C*. On this basis, there were nine dyads in each of the two data groups, of which only two dyads in each case were in reverse order of dominance (binomial tests: $P=0.09$ in each case, $n=9$). Combining probabilities by the method due to Fisher¹¹ gives $\chi^2=9.632$, which, with $2k=4$ degrees of freedom, is significant ($P<0.05$), indicating that order of conception tends to follow order of dominance ranks.

The precise *modus operandi* of this process is uncertain, but suspicion rests strongly on the likelihood that harassment during oestrus results in a disruption of the female's ovulatory cycle, either by causing it to be anovulatory or by precipitating spontaneous abortion of the ovum before or soon after fertilisation. Two lines of evidence support this interpretation. First, no differences could be found between dominant and subordinate females in either the rates of mounting or the frequencies of ejaculation received from their males. Furthermore, no female was ever observed to actively interfere with the solicitations or copulations of other females. Second, there is circumstantial evidence from captive studies of both *Theropithecus* and *Papio* that intense aggression and/or stress can result in the sudden, premature termination of oestrous cycles^{10,12}.

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Assessment of egg load by *Pieris brassicae* (Lepidoptera: Pieridae)

IF offered a choice of cabbage or *Reseda* the large white butterfly (*Pieris brassicae*) first lays a certain number of eggs on the former food-plant and then oviposits on *Reseda*. We have found that several cues enable the butterfly to assess the egg load present on a plant or leaf, and at the appropriate moment switch to the previously less acceptable species. Overloading is thus avoided. Feeding

larvae and damaged foliage are also deterrents to oviposition.

Egg-laying females (of the Cambridge and Oxford strain of *P. brassicae*¹), confined in net-covered cages, were offered a choice of two cabbage leaves from the same plant, matched for age, position on the stem, size, shape and texture. Damaged leaves and those bearing signs of mildew, aphid or slug infestation were discarded. One whole plant (with a control) was offered on which *P. rapae* had oviposited. The types of leaves used, with their treatment, are shown in Table 1. To avoid position effects, leaves were transposed periodically.

We found that *P. brassicae* lay a reduced number of eggs on leaves bearing their own eggs; model plastic eggs; crushed egg fluid; body fluids pressed from the tips of

egg fluid or body fluids, or from which eggs had been removed, indicates that airborne cues associated with the presence of eggs are appreciated from a distance.

Similar results were obtained with recently damaged leaves and crushed leaf extract painted on the surface. The reduction in the number of seedlings after contact with experimental leaves carrying a heavy egg-load, or smeared with egg fluid (Table 2), indicates that chemoreception through the tarsi⁴ also occurs.

The relative increase in the number of small egg batches (1–10) and the associated paucity of large batches (60 upwards) and a lack of orderly linear arrangement of eggs on experimental leaves, suggests that the female experiences disturbing or discouraging elements while in the act of laying, some of which may be perceived through the tip of

Table 1 *P. brassicae* ovipositing in experimental situations on cabbage leaves

Condition of leaf	No. of trials	No. of eggs trials	Remarks
{ Egg laden	45	1,129	13 leaves without eggs; none with > 150 eggs
{ Clean		6,913	No leaves without eggs; 16 with > 150 eggs
{ Smeared with <i>P. brassicae</i> egg fluid	6	317 (7 batches)	3 leaves without eggs
{ Smeared with distilled water		1,410 (34 batches)	No leaves without eggs
{ <i>P. brassicae</i> eggs brushed off surface	11	858 (25 batches)	1 leaf with under 10 eggs; none with 150 eggs
{ Surface brushed only		3,199 (93 batches)	All leaves with over 10 eggs; 1 leaf with > 150 eggs
{ With yellow model eggs attached	9	70 (4 batches)	The ♀ sometimes investigates model egg batches by extending the proboscis and drawing it back and forth over the surface like a bow across a violin
{ Clean		750 (68 batches)	
{ With first instar larvae feeding	4	140 (14 batches)	Contrast would have been greater on a clean leaf without extract
{ Clean (treated with crushed cabbage extract in 2 trials)		750 (68 batches)	
{ Damaged by feeding larvae	8	628 (18 batches)	
{ Undamaged		2,663 (74 batches)	
{ Smeared with tip of ♀ abdomen	8	234 (15 batches)	The leaf may have also been contaminated with egg fluid, haemolymph, meconium and fluid from the gut
{ Smeared with distilled water		2,880 (67 batches)	
{ Red cabbage (red drumhead cultivar)	2	40 (3 batches)	
{ Green cabbage (greyhound cultivar)		2,008 (58 batches)	
{ White (heart) leaf from same plant as control	2	313 (13 batches)	Larvae only very rarely metamorphose when reared on cabbage 'hearts'
{ Green leaf (flower of spring cultivar)		2,658 (84 batches)	
{ Old (growing)	3	874 (19 batches)	This also applies if whole young plants are matched against older plants
{ Young (growing)		1,008 (42 batches)	

female abdomens; feeding caterpillars; and leaves from which egg batches had been removed. They were not inhibited by larvae placed on a leaf surface, by model caterpillars or by an airstream with larval odour. The most powerful deterrents were feeding larvae and certain plant extracts² (Tables 1 and 2).

The butterfly's response to yellow plastic eggs or yellow patches painted on the leaves (a single trial) indicated that visual cues contribute to the selection of suitable leaves. Similarly the repellent effect of the eggs was reduced if they were dyed green. Vision may also play a part in the rejection of leaves on which larvae are feeding or which display old damage, holes and emarginations³.

The reduction in number of aerial visits (without leaf contacts) to plants and leaves bearing hidden eggs, crushed

the abdomen. This was also intimated by the behaviour of females attempting to lay on paper leaves (Table 2).

The colour and shape of eggs, if visible to the approaching butterfly (Table 1) represent one aspect of the deterrent, but our experiments indicate that an airborne pheromone-like emanation associated with the egg is the most important factor discouraging the fluttering female. After the removal of egg batches, leaves could still inhibit oviposition (Table 1), suggesting that the female, while laying, adds a deterrent marker to the eggs, either incorporated in the cement (which is difficult to remove from the leaf surface) or consisting of a discrete secretion of the accessory glands associated with the reproductive organs or genitalia, or the ovaries themselves.

Experiments using the small white (*Pieris rapae*) and

the monarch (*Danaus plexippus*) both of which lay eggs singly, suggest that a similar mechanism in these butterflies limits overloading of the host plant (Table 3). Whereas the large white chiefly depends on tarsal contact for testing the suitability of the leaf, the small white uses the tip of the abdomen, while the ovipositing monarch generally taps the leaf surface with the antennae after settling, although vision seems to have a role where larval infestation is concerned. W. W. Brandt (personal communication) has seen birdwings (Ornithoptera) in New Guinea apparently searching *Aristolochia* vines visually (starting from the bottom and working upwards) and flying off should they discover the

and around the eggs after oviposition and in ovarian tissue (but not in accessory gland extract) is said to elicit host area searching in an egg-larval parasitoid⁷. In the case of the cherry fruit fly (*Rhagoletis cerasi*) and related species^{8,9}, marking of the host plant is affected by active spreading of the repellent across the fruit surface by the ovipositor. In *P. brassicae* it seems that the pheromone-like repellent is excreted on to the leaves or, as in *Heliothis*, is present in the tissue or covering of the egg itself.

It was not surprising that *P. brassicae* responded more positively to feeding larvae than to its own eggs, since such a threat to the food source is immediate and certain. It

Table 2 Behaviour of ovipositing *P. brassicae* and evidence of airborne and contact cues

Cabbage leaf treated with plant extracts or egg material	No. and duration (h) of trial	Aerial visit without contact	Visits with leaf contacts*	Settling on leaf (wings folded but no oviposition)	No. of eggs
{ Repellent plant extract (5 species tested)		125	27	15	136
{ Cabbage leaf extract	7 trials				
	8	243	47	48	812 (+14 batches NC)
{ Attractant plant extract (3 species tested)		16	35	23	271 (+9 batches NC)
{ Cabbage leaf extract	3 trials				
	5	18	41	21	175 (+9 batches NC)
{ <i>Cheiranthus</i> extract (containing attractant and repellent)		59	NC	NC	26
{ Cabbage leaf extract	10 trials				
	10	168	NC	NC	602
{ <i>P. brassicae</i> eggs on cabbage leaf		NC	8	Nil	Nil
{ Clean cabbage leaf	1 trial				
	1	NC	33	3	2 egg batches
{ Crushed <i>P. brassicae</i> eggs on upper surface of cabbage leaf		24	NC	NC	2 egg batches
{ Cabbage leaf extract	2 trials				
	2	48	NC	NC	6 egg batches
{ <i>P. brassicae</i> eggs brushed off surface of cabbage leaf		NC	14	2	30 (2 batches)
{ Cabbage leaf (surface brushed)	2 trials				
	3	NC	11	Nil	270 (14 batches)
{ <i>P. brassicae</i> eggs removed from surface of cabbage leaf		38	NC	62	893 (25 batches)
{ Clean leaf (surface brushed)	5 trials				
	13	43	NC	116	2,366 (76 batches)
{ Green paper leaf + distilled water on surface		69	NC	NC	Nil
{ Green paper leaf + cabbage leaf extract	2 trials				
	2	82	NC	NC	4 ♀♀ ovipositing† 1 batch

NC, not counted.

*The leaf was touched with the feet or wings but fluttering continued until the butterfly flew away.

†These females went through all the motions of laying egg batches but only in one case actually extruded eggs. The small white butterfly (*P. rapae*) regularly tests the leaf with the tip of the abdomen and then decides whether or not to lay, but this is unusual for *P. brassicae*.

presence of an egg on a leaf. If such an egg is removed, the next female searcher will lay on the plant.

Similar undetected deterrents associated with eggs and feeding larvae may be widespread among the Lepidoptera. This could explain the development by some plants, such as *Passiflora*, of faked eggs³ and larva-like stipules⁵. Such a response to heavy butterfly predation assumes visual assessment by the laying female. It would be interesting to investigate the scent of these plant-part mimics, especially the faked eggs.

Repellent markers have not been recorded in the Lepidoptera before, but have been described in entomophagous parasites as a means by which the female prevents overparasitisation of the host⁶. A chemical associated with the eggs of the noctuid moth *Heliothis virescens*, present on, in

was also to be expected that deterrents found in lethal food plants (for example, *Cheiranthus cheiri* (wallflower) and *Erysimum* (treacle mustards) (refs 2 and 10 and unpublished results)) which also contain the usual cruciferous oviposition and feeding stimulants, would be both powerful and decisive in their action. Moreover, the quality of the deterrent is marvellously suited to the large white butterfly's lifestyle, for it neither inhibits nor reduces egg laying too drastically. By urging the female, fluttering among the cabbages, to 'try her luck' just a little further on, it ensures the dissemination of her progeny on suitable foliage.

We thank Howard Hinton for help with the literature, Brian Gardiner and Claude Rivers for replenishing our stocks of butterflies and for useful advice, and Bob Ford and J. Meerman for technical assistance.

Table 3 Experiments with ovipositing *P. rapae* and *D. plexippus*

Alternatives offered to <i>P. rapae</i>	Duration of experiment	No. of eggs	Remarks
Leaf of green cabbage (greyhound)	4 trials	105	—
Leaf of red cabbage (red drumhead)	1 h	12	—
Cabbage plant in pot	12 h*	254	—
<i>Tropaeolum</i> plant in pot		196	Eggs also laid on flowers
11-leaved clean cabbage plant	12 h*	245	—
13-leaved egg-laden cabbage plant		129	—
Single clean cabbage leaf	(a) 4 trials 30 min	(a) (b) 60 187	—
Single egg-laden cabbage leaf	(b) 1 trial 3 h	(a) (b) 14 116	—
Clean cabbage leaf	2 h	86	52 contact visits
Cabbage leaf with 80 eggs crushed on underside		21	25 contact visits
Clean cabbage leaf	1 h	46	—
Leaf with batches of <i>P. brassicae</i> eggs crushed on surface		20	—
Clean cabbage leaf (underside brushed)	1 trial 15 min	66	♀♀ very keen to lay and clean leaf became overcrowded
Cabbage leaf with 12 ♂♂ abdomens lightly brushed on underside		45	—
Small clean cabbage leaf (lightly brushed on underside)	3 trials 40 min	133	—
Small leaf from which 90 eggs were lightly brushed off underside		53	—
Alternatives offered to <i>D. plexippus</i>			
<i>A. curassavica</i> clean plant	3 trials	281	♀♀ near end of egg laying
<i>A. curassavica</i> egg-laden	30 h	243	—
<i>A. curassavica</i> clean plant	3 trials 12 h	112	♀♀ near end of egg laying
<i>A. curassavica</i> with feeding larvae		40	—

In the absence of leaves, *P. rapae* will oviposit on objects in the cage whereas *P. brassicae* dies without laying eggs.

*When the favoured leaf becomes egg laden the contrast declines.

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Tumours and viruses in mice injected with plutonium

THE α -particle emitting plutonium is, at present, the bone-seeking element of greatest public concern. For man, however, the best evidence of damage from α -particle emitters in bone concerns radium, for which the pattern of deposition differs from that of plutonium. We report here an experiment designed to compare the effects of plutonium in mice with those of radium, previously reported^{1,2}.

The specific questions we considered were (a) Do plutonium-induced tumours contain viruses? (b) Does sex affect the viral status or incidence of tumours? (c) Are the tumours similar to those induced by radium? (d) Are they variable with dose and time, or different in radiation chimaeras³.

The solution injected intraperitoneally was minimally polymerised plutonium (predominantly ²³⁹Pu) citrate⁴ passed through a 25-nm Millipore filter.

The design of the experiment and distribution of tumours in each of eight separate groups of animals are shown in Fig. 1. No controls are included in Fig. 1, but for 15 CBA/H female controls carefully examined earlier, and hundreds of normal CBA/H mice maintained until death but less rigorously examined, no osteosarcomas were observed. The median lifespans of untreated male and female mice are about 24–28 months beyond the age of 3 months, when treated mice were injected. These figures differ little from those of CA/H mice injected with 10 nCi ²³⁹Pu (22 and 26½ months, respectively; Fig. 1).

In chimaeras CBA T6T6/A, 9/20 males and 14/20 females developed osteosarcoma after 100 nCi ²³⁹Pu. Genetic tests by passage³ of these tumours and one skeletal haemangiosarcoma confirmed that they originated from host tissue.

In natural mice given 100 nCi, leukaemias were observed in the same number of animals as osteosarcomas, 2/10 males and 5/10 females. After 30 nCi, osteosarcoma occurred in 4/10 male and 8/10 female mice. After 10 nCi, no osteosarcomas were observed in male mice but 9/10 died of other tumours including four carcinomas, two fibrosarcomas, two reticulum-cell sarcomas and one possible haemangiosarcoma: in females, however, osteosarcoma was the commonest tumour (6/10), accompanied by carcinoma in three, and two mice died early with reticulum-cell sarcoma.

Osteosarcoma is more readily induced in female than male mice, owing to the response of murine bone to oestrogens⁵ (though in man the natural incidence of osteosarcoma is higher in males than females^{6,7}).

The high incidence of tumours other than osteosarcoma, though unexpected, could agree with observations for rats inhaling soluble ²³⁸Pu (ref. 8) and rats injected with various bone-seeking radionuclides⁹. Regarding leukaemia, though a small incidence of myeloid leukaemia¹⁰ and 'leukoses of the reticulosis type'⁹ occurred in rats given plutonium, it has not been reported in mice. In CBA with a natural incidence of about 1% (refs 11, 12), seven cases out of 20 at risk of generalised lymphomatosis (excluding thymus) with lymphoblasts in peripheral blood was striking. Leukaemias occurred earlier than osteosarcomas. Also, there is a suggestion of an increase of early reticulum-cell sarcoma in animals given 10 nCi (the incidence in normal CBA mice is 5–10% (refs 2, 13) and of miscellaneous malignancies after 10 and 30 nCi, many at times later than the mean time for appearance of osteosarcoma (and so reminiscent of the late onset of specific carcinomas of cranial air sinuses in human radium cases, notably those with lower burdens of radium¹⁴). The late onset may be related to the long retention of radioactivity and immunological imbalance.

None of the chimaeras, which have perhaps an even lower

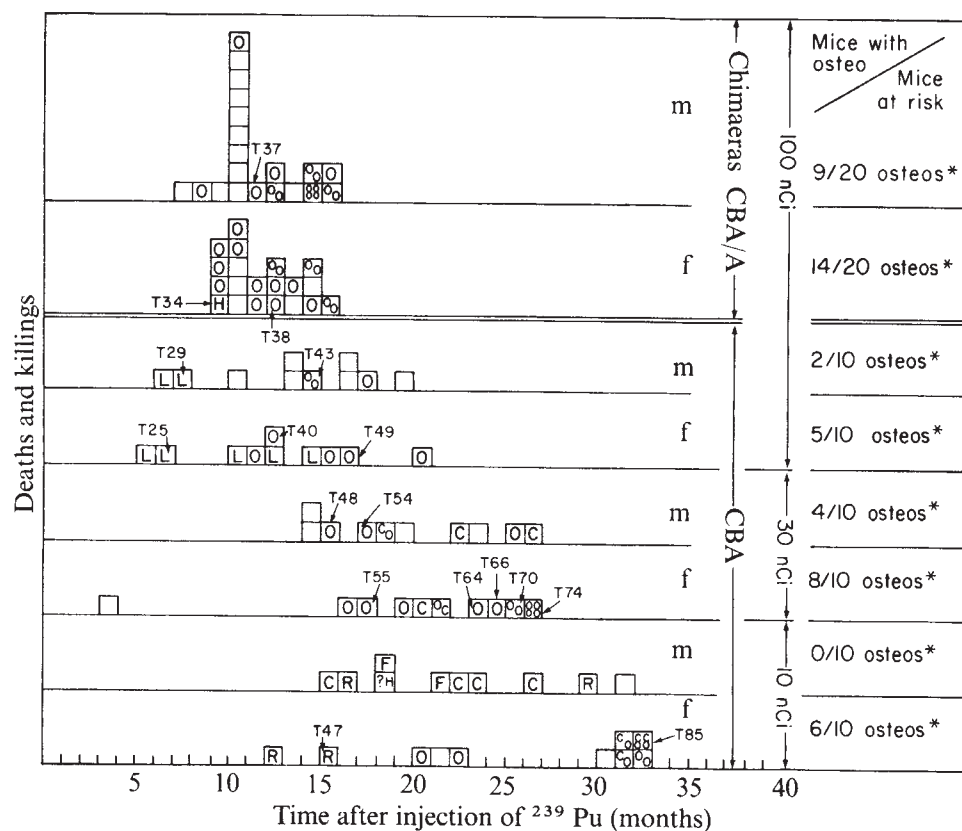


Fig. 1 Design and results of the experiment involving i.p. injection of CBA/H mice and CBA T6T6/A radiation chimaeras with ^{239}Pu citrate. Each mouse is represented by an individual box. This is plotted with time after injection of varying doses of ^{239}Pu citrate when the mouse either died or was killed. The types of tumours are indicated: O, osteosarcoma; H, haemangiosarcoma; L, leukaemia; C, carcinoma; F, fibrosarcoma; R, reticulum-cell sarcoma. Multiple entries indicate multiple tumours. Samples examined by electron microscopy are designated by the letter T followed by a number, for instance, T37. *osteosarcoma; m, male; f, female.

incidence of all forms of lymphoma¹⁵ than natural mice, developed leukaemia after 100 nCi. This may be due to a somewhat lower uptake of plutonium by bone marrow in chimaeras¹⁶. Such chimaeras, however, have a low incidence (1/69) of generalised lymphoma induced by β rays of ^{90}Sr - ^{90}Y on donated haematopoietic tissue¹⁷. One might expect the 1,000 rad of whole-body irradiation given to the chimaeras to alter the normal cellular and humoral environment but not to impair the expression of virus, rather the reverse¹⁸.

The median lifespans of animals treated with 10 nCi (24 months) 30 nCi (20 months) and 100 nCi (13 months for CBA/H mice and 11 months for chimaeras) were shorter than for untreated controls (26 months). Malignant disease, varying in type between groups, was a major cause.

Table 1 lists the viral status of tumours examined electron

micrographically. In osteosarcomas taken from chimaeras injected with 100 nCi ^{239}Pu , more virus particles were seen than in the other tumours. In 16 out of 19 tumours examined, virus was seen, and 'virus-like' particles were seen in one other tumour. No virus was observed in either of two tumours examined from animal T85. Figure 2 is an electron micrograph demonstrating the typical type-C particles. Viruses were commonly found either lying within cytoplasmic vesicles or budding from endoplasmic reticulum associated with degenerate cells. No difference could be seen in morphology of viruses in samples taken from different tumour types.

In the series no tissue taken from normal ribs of three CBA/H tumour-bearing animals showed any evidence of virus even after extensive search, though occasional viruses were seen in control animals of an earlier study¹.

Table 1 Viral status of ^{239}Pu -induced tumours

Dose per mouse (nCi)	Strain	Sex	ANL no.	Tumour type	Site of tumour	Time of appearance (months)	Virus present or absent
100	Chimaeras	Male	T37	Osteosarcoma	Sacrum	11	+
		Female	T34	Haemangiosarcoma	Sacrum	9	+
			T38	Osteosarcoma	Sacrum	12	+
	CBA	Male	T29	Lymphoma	Lymph node	7	+
			T43	Osteosarcoma	Right ilium	14	+
				Osteosarcoma	Thoracic vertebra 12		+
		Female	T25	Lymphoma	Lymph node	6	-?
			T40	Osteosarcoma	Ischio pubis	12	+
			T49	Osteosarcoma	Humerus	16	+
			T48	Osteosarcoma	Lumbar vertebra 5	15	+
30	CBA	Male	T54	Osteosarcoma	Patella	17	+
		Female	T55	Osteosarcoma	Parosteal to humerus	17	+
			T64	Osteosarcoma	Cranium	23	+
			T66	Osteosarcoma	Sacrum	24	+
			T70	Osteosarcoma	Sacrum	25	+
			T74	Osteosarcoma	Scapula	26	+
		Female	T47	Reticulum-cell sarcoma	Liver	15	+
			T85	Osteosarcoma	Rib	32	-
				Carcinoma	Lung		-
							-

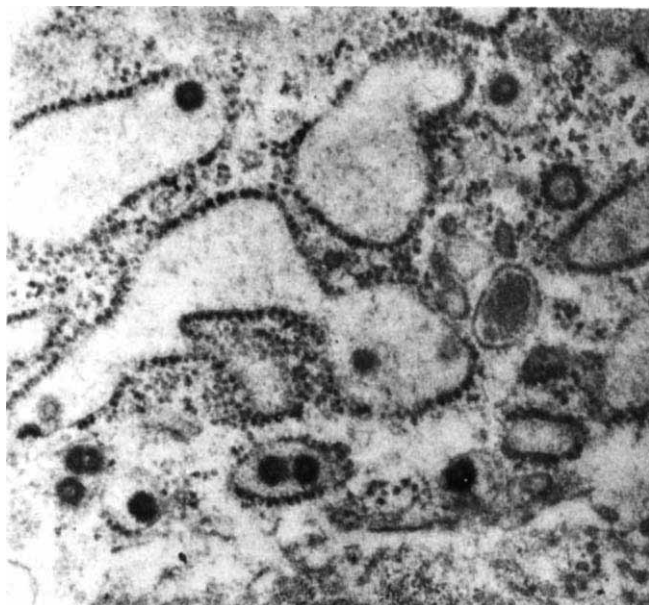


Fig. 2 Electron micrograph of a portion of tissue taken from an osteosarcoma (T54) showing viruses lying within cytoplasmic vesicles or budding from the endoplasmic reticulum ($\times 47,600$).

The particles appear identical with those observed in osteosarcomas from CBA/H animals injected with ^{226}Ra (ref. 1). This does not allow us, however, to implicate virus as the aetiological agent. Tumour tissue may merely provide a favourable environment in which virus can replicate sufficiently to be above the threshold of detection by electron microscopy. Three lines of evidence from current work in progress indicate that viral action need not be involved in radiation-related tumorigenesis. First, animals subjected to a procedure of immunisation with homogenates of tumour tissue and subsequently injected with ^{226}Ra develop osteosarcoma to about the same extent and in the same time as unimmunised controls¹⁹. Second, a single α particle directly hitting the nucleus may be insufficient to kill the cell²⁰, contrary to what some have previously supposed. Hence, survival of some cells with alteration of DNA is probable after a direct hit on the nucleus, and may lead to malignancy without the intervention of virus. Third, cells irradiated in culture with α particles have been transformed²¹, in a manner similar to that shown by other workers using γ rays²² and chemical agents²³, where transformation was shown to be independent of virus expression.

Our results need confirmation with larger groups of animals and smaller doses of plutonium (the lowest dose used led to a concentration about a thousandfold greater than that occupationally permissible to man).

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RD-114 and feline leukaemia virus genome expression in natural lymphomas of domestic cats

THE widespread distribution of endogenous type C viral genetic sequences in many different vertebrate species^{1,2} is now well established. The biologic implications of the presence of such endogenous type C viral genomes, especially with respect to normal and neoplastic growth, are at present of considerable interest³. The domestic cat (*Felis catus*) offers a unique model system for approaching this question, because of its ready availability, outbred nature and relatively short lifespan. Moreover, the genetic information of its endogenous xenotropic virus (RD-114) is distinct⁴ from that of feline leukaemia virus (FeLV), a horizontally transmitted, infectious type-C virus of this species. RD-114 virus⁵ is generally non-infectious for cat cells⁶ and its pathogenicity is undetermined, whereas FeLV is known to be leukaemogenic under natural⁷ and experimental⁸ circumstances. The genetic information of RD-114 has been demonstrated in the DNA of domestic cats^{9–11} and their close relatives, but not in more distantly related species of the genus *Felis*¹². Both transcriptional¹³ and translational¹⁴ products of the endogenous RD-114 viral genome are also found in certain instances in the absence of complete virus in normal cat tissues. FeLV and RD-114 gene expression at the transcriptional and translational levels in domestic cats has been examined here in relation to naturally occurring lymphoma. Results indicate a significantly higher level of RD-114 transcription and translation in most lymphoma tissues compared with normal tissues. Although FeLV expression is also pronounced in lymphomas, this generally reflects the presence of infectious FeLV. In general, however, only the lymphomas from the younger cats show significant levels of FeLV gene expression, whereas RD-114 expression is high in lymphomas of both young and old cats. Although the relationship of this enhanced expression of the RD-114 genes to the neoplastic process is unknown, these findings indicate that certain functions of the endogenous RD-114 viral genome are closely associated with the development of lymphoid tumours in the cat.

Nucleic acid and antigenic differences between RD-114 and FeLV made possible the measurement of the expression of viral information for both viruses in the same tissues at the RNA and structural protein levels. The transcription of virus-specific RNA in the tissues was determined by molecular hybridisation using ³H-labelled single-stranded DNA products (³H-cDNA) complementary to viral nucleotide sequences and the S₁ nuclease method of assay. The levels of the 30,000 molecular weight major structural protein (p30) of both viruses were determined on the same tissues by competition radioimmunoassays. Sixty histologically normal tissues from 47 cats free from cancer and 55 lymphoma tissues from 31 lymphomatous cats were assayed for the RNA and p30 of RD-114 and FeLV. The normal tissues examined included spleen, liver, and

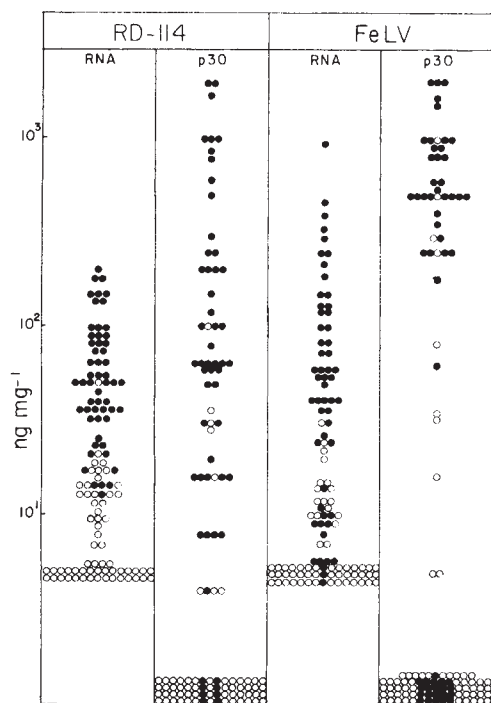


Fig. 1 Measurement of RD-114- and FeLV-specific RNA and p30 in normal and lymphoma tissues of cat. Viral RNA concentrations were determined by molecular hybridisation of tissue RNA with viral ^3H -cDNA using the S_1 nuclease assay. Total tissue RNA was prepared following a modification of a published procedure²⁰. ^3H -cDNA was synthesised using a modified procedure of Benveniste and Scolnick²¹. Briefly, purified²² RD-114 virus or FeLV (Gardner-Arnstein strain) grown in the human rhabdomyosarcoma (RD) cell line³ was incubated at 37 °C for 4 h in a reaction mixture containing 0.024 mM ^3H -dTTP (92.9 Ci mmol⁻¹), 3 mM each of dATP, dGTP, dCTP, 20 $\mu\text{g ml}^{-1}$ actinomycin D, 60 mM KCl, 6 mM $\text{Mg}(\text{CH}_3\text{COO})_2$, 5 mM dithiothreitol, 40 mM Tris-HCl, pH 8.0, and 0.02% Nonidet P-40. ^3H -cDNA was shown to contain representative transcripts by its ability to protect ^{32}P -labelled viral RNA from digestion by RNase A and T₁ (ref. 23). RD-114 ^3H -cDNA hybridised with 78% of the viral RNA at a ^3H -cDNA to ^{32}P -viral RNA ratio of 1.0. FeLV ^3H -cDNA protected 67% of FeLV viral RNA at the same ratio. The viral RNAs were prepared as described previously²⁴. The specificity of the probe was shown by the ability of RD-114 cell and viral RNA to protect 95% and 100%, respectively, of the RD-114 ^3H -cDNA from S_1 nuclease digestion, whereas only 3% and 2% of the probe was protected by FeLV viral- and FeLV-infected RD cell RNA. FeLV ^3H -cDNA hybridised at 51% and 56% with homologous infected cell RNA and viral RNA, respectively, whereas only 2% and 5% of the ^3H -cDNA hybridised to heterologous RD-114 cell and viral RNA. All hybridisation reactions were carried out in 10 μl containing 0.3 M NaCl, 0.03 M Na citrate, 0.05% SDS, 0.02% diethylpyrocarbonate, 500–800 c.p.m. ^3H -cDNA (7.7×10^3 c.p.m. μg^{-1}) and various amounts of tissue RNA in sealed capillary tubes at 68 °C for 16 h. Hybridised fractions of ^3H -cDNA were determined by TCA-precipitable radioactivity after digestion with 35 $\mu\text{g ml}^{-1}$ S_1 nuclease in a reaction mixture containing 0.17 M ZnCl₂, 27 mM Na acetate (pH 4.5), 0.11 M NaCl and 17 $\mu\text{g ml}^{-1}$ heat-denatured salmon sperm DNA for 70 min at 45 °C. Concentration of RNA $\times$ time of incubation (C_t) values up to about 3×10^4 (corrected to a monovalent cation concentration of 0.18 M; ref. 25) were generated with tissue RNA and the concentration of virus-specific RNA per mg cell RNA was determined by comparison of the C_t value at 20% level of hybridisation with that obtained using viral RNA. The lower limit of sensitivity varied between 5–10 ng virus-specific RNA per mg cell RNA, depending on the amount of tissue RNA available for the assays. Tissues with undetectable levels of RNA were arbitrarily listed as 5 ng per mg cell RNA. The thermal denaturation of hybrids formed between the RNA from two tumour tissues and the ^3H -cDNA probes of RD-114 and FeLV was determined for comparison with the corresponding T_m values obtained with RD-114- and FeLV-infected cell RNA. The hybrids exhibited a similar T_m (81.5 °C in 0.3 M NaCl, 0.03 M Na citrate, 0.05% SDS), indicating that most of the viral RNA generated in the tissues was closely related to the RNA produced in the virus-infected cell cultures. The values

kidney; most of the lymphomas were located in the spleen, lymph nodes, kidney, and a few were in the liver, thymus, and other tissues. The presence of infectious FeLV was determined in each cat by the indirect immunofluorescent antibody test for cytoplasmic FeLV p30 antigen in leukocytes and platelets of fixed peripheral blood or bone marrow smears. This test has proved to be a reliable index of systemic infection with FeLV (ref. 15).

The results summarised in Fig. 1 demonstrate that the levels of expression of RNA and p30 of either RD-114 or FeLV generally segregate into two distinct groups, one representing normal and the other lymphoma tissues. The majority of values for the normal tissues cluster below 10 ng per mg cell RNA or protein. In contrast the levels of both RNA and p30 of the endogenous xenotropic virus, RD-114, were considerably higher in the lymphoma tissues. In general, a good correlation was observed between the presence of RD-114-specific RNA and p30 in lymphoma tissues, with the exception of a few tissues which exhibited low but detectable levels of RNA expression in the absence of p30. This finding suggests that, although transcription of the RD-114 genome may occur in certain feline lymphomas, the entire genome is not necessarily translated. Many but not all of the lymphomas also exhibited elevated levels of FeLV RNA and p30 as compared with the normal tissues. Interestingly, most of the FeLV p30-negative lymphomas originated from older cats. As shown in Fig. 2, in which animals ≤ 5 yr of age have been classified as younger cats and those ≤ 7 yr as older cats, a general correlation was observed between the age of cats and virus gene expressions in their lymphomas. Whereas RD-114 RNA and p30 were expressed in the lymphomas of both younger and older animals, FeLV expression seemed to be most prevalent among cats ≤ 5 yr of age. All but two lymphomas from cats of this younger group contained FeLV RNA (≥ 25 ng mg^{-1}) and high p30 levels (≥ 180 ng mg^{-1}). Moreover, there was an excellent correlation between elevated p30 levels and systemic infection with FeLV in these younger cats¹⁶. Thus, the detection of FeLV p30 and RNA expression in these animals probably reflected the presence of horizontally transmitted infectious FeLV. The vast majority of the lymphomas from the older cats, on the other hand, did not contain detectable FeLV p30, although some exhibited levels of RNA significantly higher than those found in normal tissues. The presence of RD-114 gene products and low or no expression of FeLV genes were consistently observed in multiple lymphoma tissues of individual old cats (data not shown).

The geometric mean values of the data and the statistical significance of the observed differences between the normal and lymphoma tissues from old and young cats are summarised in

(ng per mg cell protein) of p30 were determined by group-specific competition radioimmunoassays using ^{125}I -labelled p30 antigen of FeLV and RD-114 as probes²⁶. Cell extracts were prepared by homogenisation of tissues in equal volumes of 0.01 M Tris-HCl, pH 7.8, 0.1 M NaCl, 0.5 mM EDTA and 0.5% Triton X-100, sonication for 1 min by use of a Biosonic II sonic oscillator, and clarification by centrifugation for 1 h at 100,000g. Antisera prepared in goats to either FeLV or RD-114 and serial twofold dilutions of competing tissue extract were incubated at 37 °C in 0.15-ml reaction mixtures containing 10 mM Tris-HCl, pH 7.8, 10 mM EDTA, 0.4% Triton X-100, 1% bovine serum albumin, 0.1% normal goat serum, 10 mM NaCl, and 300 $\mu\text{g ml}^{-1}$ phenylmethane sulphonyl fluoride. About 10,000 c.p.m. ^{125}I -labelled FeLV or RD-114 p30 in 0.05 ml of the same buffer were added and incubation continued for 3 h at 37 °C and a further 18 h at 4 °C. Antigen-antibody complexes were precipitated by the second antibody technique and radioactivity in the precipitates measured in a Searle model 1285 gamma counter. Levels of p30 in extracts, expressed as ng per mg cell protein, were calculated on the basis of displacement of the competition curve for the unknown relative to that achieved with purified viral antigen. Cell protein determinations were performed according to the method of Lowry²⁷. The lower limit of sensitivity of the competition radioimmunoassay was 2 ng per mg cell protein. Tissues with undetectable levels of p30 were arbitrarily listed as 1 ng per mg of cell protein. ○, Histologically normal tissues from cats free of cancer; ●, lymphoma tissues.

Table 1 Virus-specific RNA and antigen (p30) expression in normal and lymphoma tissue of cats of different age groups

Virus	Assay	Young cats (≤ 5 yr)		<i>P</i>	Old cats (≥ 7 yr)		<i>P</i>
		Normal (35)	Lymphoma (32)		Normal (24)	Lymphoma (22)	
RD-114	RNA	7.1	46.1	<0.0005	8.4	65.8	<0.0005
	p30	1.6	48.3	<0.0005	1.3	59.0	<0.0005
FeLV	RNA	5.8	81.7	<0.0005	7.8	17.1	<0.0100
	p30	1.7	414.0	<0.0005	2.4	2.3	<0.5000

Viral RNA and p30 concentrations were (determined as summarised in Fig. 1. Values within parentheses represent the number of tissues tested. The geometric mean values listed represent ng virus-specific RNA or p30 per mg cell RNA or protein. *P* values were calculated using paired Student's *t* test of the logarithmic values of the ng mg⁻¹ data.

Table 1. The differences in the expression of RD-114 genes in the lymphoma and normal tissues, at RNA or p30 level, are highly significant ($P < 0.0005$) irrespective of the age of the cats. Equally significant is the difference in the FeLV gene expressions in the lymphomas of young cats and the normal tissues from the corresponding age group. In contrast, no significant difference was found in the FeLV expression at the p30 level between the lymphomas from old cats and the corresponding normal tissues. The data suggest, however, a significant transcription of FeLV RNA in the absence of detectable p30 in a few lymphomas of old cats. Since sequences related to FeLV in the absence of infectious FeLV can be detected in the genomes of domestic cats^{9,17,18}, this transcription could represent either expression of these endogenous FeLV sequences, or subinfectious expression of exogenous FeLV acquired, perhaps, at an earlier age. Thus, it seems that the expression of the endogenous xenotropic RD-114 virus information is closely associated with lymphomas in cats of all ages, whereas FeLV related gene expression is primarily associated with lymphomas of younger cats. The absence of FeLV gene expression in many older and a few younger lymphomatous cats confirms our earlier findings based on other

assays for FeLV (ref. 19), and suggests that, in these particular cats, spontaneous lymphoma may not be caused by FeLV. The widespread occurrence of RD-114 viral gene products in lymphomas suggests that expression of certain functions of this endogenous virus may be involved etiologically in the development of spontaneous lymphoid tumours of the cat. The alternative possibility, however, that elevated endogenous viral gene expression may be the result, rather than the cause, of neoplastic transformation cannot be ruled out by the present findings. It is our hope that elevated endogenous viral gene expression, even if not causatively involved in lymphomagenesis, may provide a logical approach towards immunoprevention.

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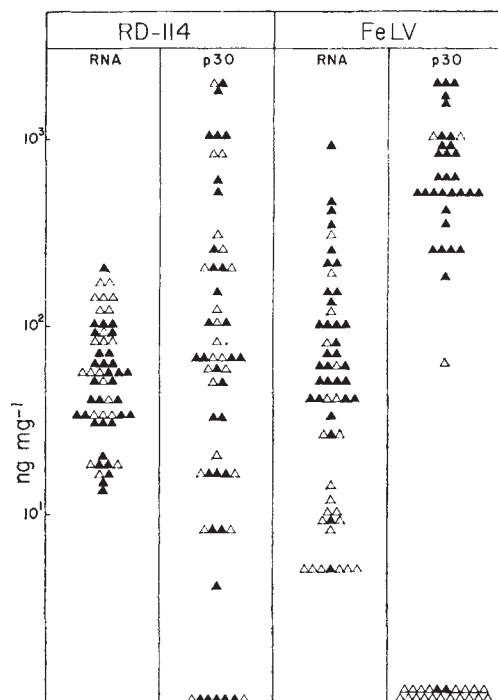
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Fig. 2 Virus-specific RNA and p30 expressions in lymphomas from young and old cats. Values were calculated as in Fig. 1. Cats ≤ 5 yr of age were designated as young and ≥ 7 yr of age as old. There were no 6-yr-old cats in this study. $\blacktriangle$, Young; $\triangle$, old cats.



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Persistence of virus-specific memory B cells in mice CNS

IMMUNOGLOBULIN (Ig) populations detected in the cerebrospinal fluid (CSF) in chronic viral infections of the central nervous system (CNS) differ from serum Ig with regard to the relative concentration of antiviral antibodies^{1–4}, their light chain ratio⁵ and their heterogeneity^{6,7}. Such observations support the idea that antibodies are produced locally in the CNS⁸. But differences between CSF and serum Ig can also be explained in terms of the selective retention and accumulation of serum antibodies after passage across the blood–brain barrier⁹. Unequivocal proof of the local antibody production requires, however, that plasma cells, or their precursor cells, be isolated from the CNS. Intracerebral inoculation of mice with 6/94, a para-influenza type 1 virus¹⁰, induces an immunologically mediated degeneration of the white matter which is related to infiltration of the brain parenchyma by mononuclear cells^{11,12}. In the study reported here, cells recovered from the brains of virus-infected mice were investigated as described previously to analyse memory B cells reactive to influenza viruses^{13,14}. Virus-reactive memory B cells, that is immunocompetent cells able to generate, on antigenic stimulation, a clone of antiviral-antibody-producing plasma cells, were demonstrated in the brains of mice injected intracerebrally with 6/94 virus but not in the brains of mice injected intraperitoneally.

BALB/c mice were inoculated intracerebrally or intraperitoneally with 6/94 virus. At various times the 6/94-reactive memory B cells obtained from brain, blood and spleen of individual donor mice were counted in an adoptive transfer system described previously¹³. Cells used for adoptive transfer were prepared as follows. Blood was withdrawn by heart puncture and red cells were lysed by incubation in NH₄Cl (ref. 15). White cells were pelleted through a cushion of calf serum¹⁵ and resuspended in a balanced salt solution (BSS). A spleen cell suspension was prepared by gentle disruption of the spleen in a tissue homogeniser¹³. To obtain brain cells the brain was removed aseptically, rinsed in cold BSS, finely diced with a tissue chopper (McIlvain, Brinkman) and resuspended in ice-cold BSS. Large brain fragments were allowed to settle and the supernatant was withdrawn and spun for 8 min at 800g at 4 °C. The pellet was resuspended in BSS and mixed with a solution of bovine serum albumin (BSA) in BSS to give a final BSA concentration of 21%. A discontinuous BSA gradient was prepared, as described by Shortman¹⁵, by underlaying a 32% BSA solution and overlaying BSS. The gradient was spun for 20 min at 3,000g. Cells that had accumulated at the interface between 32% and 21% BSA were collected, spun out of the BSA and resuspended in BSS. Between 1 and 4 × 10⁵ viable cells (Trypan blue exclusion) were recovered from a single brain. The BSA purification of the brain extract was necessary because the intravenous injection of crude brain eluate produced a high death rate in recipient mice, probably caused by microemboli. Each cell preparation was then injected intravenously into lethally irradiated syngeneic recipient mice¹³. Splenic fragments of the recipient mice were cultured individually (50–70 fragments per spleen) and analysed by radioimmunoassay (RIA)¹⁴ for the production of antibodies to 6/94 virus after secondary stimulation *in vitro* with 6/94. In these conditions, the number of antibody-producing splenic fragments has been shown to be correlated with the number of transferred precursor B cells¹³.

The results of several adoptive transfer experiments with cells from the brain of mice at various times after intracerebral or intraperitoneal inoculation with 6/94 are shown in Tables 1 and 2, respectively. Two points are noteworthy. First, 6/94-reactive memory B cells could be demonstrated in the brain cell eluate from

Table 1 6/94-reactive memory B cells from mice inoculated intracerebrally with 6/94 virus

Weeks after i.c. infection of donor mice*	No. of anti-6/94 antibody-producing splenic fragments per 10 ⁵ transferred cells		
	Brain	Circulation	Spleen
4	0.9 (2/2.2)	0.07	0.41
5	10.0 (5/0.5)	0.25	1.0†
7	0.8 (2/2.5)	0.73	0.60
9	< 0.2 (0/4.8)	0.82	2.0†
18	0.6 (3/4.8)	0.70	0.80
31	(10/half brain)	ND	ND
34	(None/half brain)	< 0.2	1.10

To establish that no background antibody production occurred with any given batch of recipient mice, each experiment included fragment cultures from lethally irradiated mice that had not received adoptive transfer of cells. All these control cultures in the experiments included in Tables 1 and 2 were negative.

* BALB/c mice were inoculated intracerebrally (i.c.) with approximately 10^{6.3} EID₅₀ of 6/94 and were killed at the times indicated and cells prepared for use in the adoptive transfer. Antiviral-antibody-producing splenic fragments were counted 12 and 15 d after their secondary stimulation *in vitro* with 6/94.

† There were too many transferred cells for an accurate determination of precursor cell frequency. ND, not determined. Figures in parentheses show total numbers of foci observed per total number of transferred cells × 10⁵.

all but two of the intracerebrally inoculated mice. Although the absolute numbers of detected foci per experiment was small (because of the paucity of cells recovered from brain) the relative frequency (per 10⁵ transferred cells) of 6/94-reactive memory B cells was, in the positive experiments, of the same magnitude or higher than in circulation or spleen. Second, virus-reactive memory B cells seemed to persist in the brains of individual mice for at least 4–7 months after intracerebral inoculation.

Our results (excluding the final two lines of Table 1), can be summarised to show that antiviral antibody was produced by 12 splenic foci obtained after transfer of 14.8 × 10⁵ brain eluate cells after intracerebral inoculation; conversely, no antibody was produced by any of the spleen fragments after transfer of 9.7 × 10⁵ brain eluate cells after intraperitoneal inoculation. It is thus evident that 6/94 memory B cells were detected in the brain eluate only after intracerebral inoculation of donor mice. Given that the number of 6/94-reactive memory B cells in the circulation was approximately the same after inoculation by both routes (Tables 1 and 2), and assuming that the degree of contamination of the brain eluate cells by circulating blood cells was the same in both intracerebrally and intraperitoneally inoculated mice, we conclude that the 6/94 memory B cells in the brain eluate cell preparation of intracerebrally inoculated mice were previously localised in the brain parenchyma and did not represent contaminating blood cells. This is supported by the results of experiments done 4 and 5 weeks after intracerebral inoculation when, on adoptive transfer, the brain eluate cells produced 10–40 times (Table 1) more splenic foci than did the same number of white blood cells of the given donor mice.

In the analysis reported here, the comparison was between the number of 6/94-reactive precursor B cells and the total number of

Table 2 6/94-reactive memory B cells obtained from mice inoculated intraperitoneally with 6/94 virus

Weeks after i.p. infection of donor mice*	No. of antibody-producing splenic fragments per 10 ⁵ transferred cells		
	Brain	Circulation	Spleen
3	< 0.4 (0/2.4)	0.27	ND
10	< 0.3 (0/2.9)	0.58	ND
18	< 0.3 (0/2.8)	0.44	1.25
34	< 0.6 (0/1.6)	0.20	0.92

See legend to Table 1.

* BALB/c mice were inoculated intraperitoneally (i.p.) with approximately 10^{7.3} EID₅₀ of 6/94. ND, not determined.

transferred cells, regardless of type. It would be more useful to correlate the number of 6/94-reactive precursor B cells with the number of transferred B cells. It is not known what proportion of the cells eluted from the brain consists of B cells, but it is presumed to be smaller than the proportion of B cells in white blood cells (approximately 20%, refs 16 and 17) or spleen cells (approximately 40%, ref. 18). Thus our data probably underestimate the actual difference in terms of absolute frequency (per transferred B cell) of 6/94-reactive memory B cells in the brain of intracerebrally infected mice as opposed to the circulation or the spleen.

This study provides the first direct evidence for the presence of virus-reactive memory B cells in brain tissue of mice with a virus-induced neurological disease. A local immune response can thus be maintained within the CNS.

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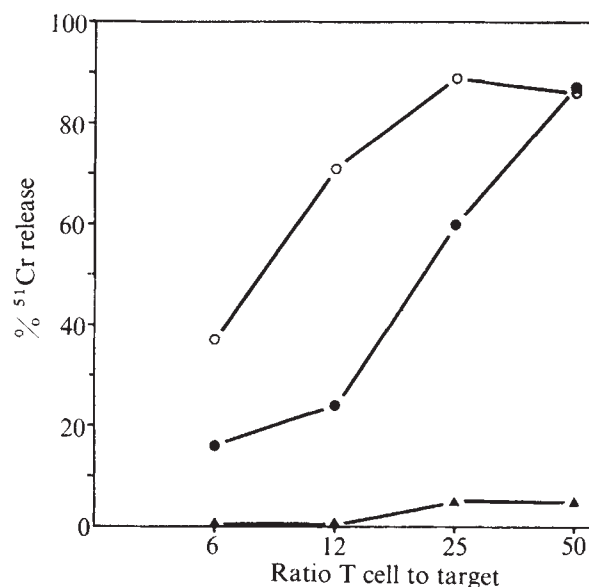


Fig 1 The F-9 teratocarcinoma cell is not lysed by alloreactive T cells specific for H-2^b antigens, expressed on MC57G tumour cells and C57BL primary peritoneal macrophages. Cytotoxic effectors were generated by secondary stimulation of memory lymphocytes from BALB/c mice with mitomycin C-treated (50 µg ml⁻¹) C57BL spleen cells¹⁵. The assay was incubated for 16 h at 37 °C and results are expressed as % specific ⁵¹Cr release.

lymphocytes sensitised against LCMV. The PCC4 line, however, has remained multipotential and can give rise to various different cell types, at least some of which show evidence of H-2 gene function (Table 1) and are attacked by the appropriately sensitised lymphocytes.

Equivalent amounts of cell-surface viral antigen are found on immunofluorescent examination⁷ of unfixed, LCMV-infected F-9, PCC4 and MC57G (H-2 positive) targets. Maximal virus-specific T-cell effector function is, however, recognised only for the MC57G cell line (Fig. 2). Apparently some PCC4 cells are lysed, but LCMV-infected F-9 cells are evidently not damaged by virus-immune lymphocytes (Fig. 2). Repeated experiments with the PCC4 targets gave extremely variable results (from 21% to 90% of the virus-specific ⁵¹Cr release found for MC57G) and, although the two series of observations have not yet been made concurrently, the extent of H-2 antigen expression on PCC4 cells may also vary considerably (from 14% to 56%). The limiting factor in virus-specific T cell-mediated immunity would thus seem to be H-2 gene expression in the target cell. This is in accord with the observation that blocking of tumour-specific effector T cells may be achieved by treating the targets (but not the lymphocytes) with anti-H-2 serum^{8,9}, and with previous experiments using trinitrophenyl (TNP)-modified F-9 cells¹⁰.

Exposure of the cell membrane to TNP might be thought to induce direct alteration of the H-2 antigen, which would obviously not occur if the antigen is absent. However, infectious virus which

H-2 gene expression is required for T cell-mediated lysis of virus-infected target cells

EFFECTOR T cells generated during the course of virus diseases of mice interact only with virus-infected target cells sharing H-2K or H-2D antigenic specificities with the mouse strain in which the lymphocytes are sensitised¹⁻³. The current interpretation⁴ of this phenomenon is that the cell membrane component(s) recognised by the T-cell receptor(s) has characteristics of both self (H-2) and non-self (virus). Phenotypic expression of H-2 genes in the target cell would thus be essential for T cell-mediated immunity. Here we test this hypothesis using two teratocarcinoma cell lines (F-9 and PCC4 derived from strain 129 mice^{5,6}) infected with lymphocytic choriomeningitis virus (LCMV). We show that the F-9 tumour cells apparently do not express H-2K or H-2D antigenic specificities (Fig. 1, Table 1), and are also resistant to attack by

Table 1 Presence of serologically-detectable H-2 antigens

Target cells	Mouse strain and H-2 type	% Positive by immunofluorescence			
		anti-H-2K ^b (33, 53, 54)	anti-H-2D ^b (2, 28, 59, 56)	anti-H-2D ^b (2, 56)	Normal C57BL
F-9	129, H-2 ^{bc}	< 1	< 1	5	4
PCC4	129, H-2 ^{bc}	42	16	30	2
MC57G	C57BL, H-2 ^b	98	ND	91	4
Spleen	129, H-2 ^{bc}	96	99	ND	32*
Spleen	C3H, H-2 ^k	27*	32*	ND	33*

Mouse alloantisera 33, 28b and D-2, reactive to the H-2 specificities shown above in parentheses, were supplied by the Transplantation and Immunology Branch, National Institute of Allergy and Infectious Diseases, NIH. Fluorescent rabbit IgG specific for mouse IgG heavy and light chains were purchased from Cappel Laboratories, Downingtown, Pennsylvania. All sera were used at a dilution of 1:10.

* Reactivity is due to Ig-bearing B cells and antibody-forming cells in the spleen population.

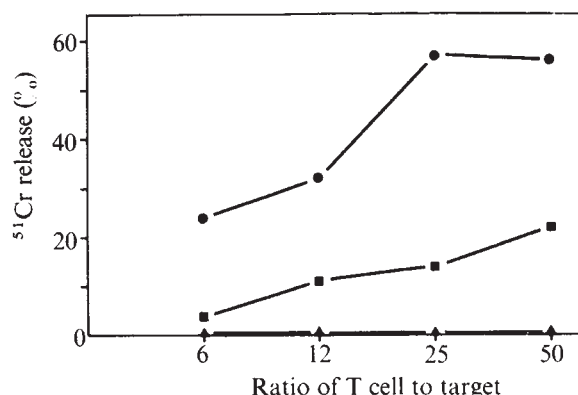


Fig. 2 The extent of specific lysis of virus-infected target cells by virus-immune effector T cells from C57BL mice injected with 1,000 LD₅₀ of WE3 LCMV 8 d previously. The assays¹⁶ were incubated for 16 h at 37 °C.

has been introduced to the cell at a relatively low multiplicity (5 LD₅₀ per cell 48 h before assay), could potentially modify cell-surface structure in other ways. Perhaps the process of virus growth causes derepression of cryptic genes within the H-2 complex, and the "virus-immune" T-cell response is directed solely against abnormally expressed H-2 antigen¹¹. Alternatively some "interaction antigen"^{12,13} may be formed, which is partly coded for by H-2 genes and partly by the viral genome. Apparently neither of these mechanisms, which could conceivably be independent of phenotypic expression of normal H-2 antigen, operates in the virus-infected F-9 cell or is obviously relevant to the variability observed with PCC4. Perhaps the necessary genes have been deleted from the F-9 tumour, although these cells express a closely-linked gene product which may be the embryonic equivalent of H-2 (ref. 14).

Use of this experimental system also establishes that absence of H-2K or H-2D cell-surface antigens in no way facilitates lysis mediated by virus-immune T cells (differing in H-2 specificity) (Table 2). It seems likely therefore that the presence of foreign H-2K or H-2D determinants does not inhibit lymphocyte function. Occurrence of virus-specific cell-mediated immunity ap-

Table 2 Absence of H-2 does not facilitate lysis of different H-2 specificity by virus-immune T cells

Immune spleen	MC57G	% ⁵¹ Cr release from virus-infected targets F-9	B10.A
B10 (H-2 ^b)	39	0	5
B10.A (H-2 ^a)	0	1	78
CBA/J (H-2 ^k)	0	0	25

Mice were injected with 1,000 LD₅₀ of WE3 LCMV 8 d previously, and spleen cells and targets were mixed in a ratio of 30:1 (ref. 16). The B10.A and CBA/J mouse strains share H-2K^k antigenic specificities. The B10.A target cells were peritoneal macrophages.

parently depends on recognition by the lymphocyte of some cell membrane component coded for by the H-2 gene complex. The possibility that H-2K and H-2D antigens function as "self-markers" in cell-mediated immunity⁴ must be considered.

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A recombinant in the major histocompatibility complex of the rat

WE report here a recombinant in the major histocompatibility complex (MHC) of the rat, and describe the properties of alloantisera directed at the recombined portions of one haplotype. The results are compatible with a structure for the rat MHC more closely resembling that of man than that of the mouse.

The inbred rat strains used were HO(PVG/c) (AgB5 = H-1^a), DA(AgB4 = H-1^a) and AO (AgB2 = H-1^a). An historical accident has led to the use of two parallel nomenclatures for the rat MHC. We use the AgB terminology of Palm¹ without prejudice to a future reconciliation with the Rat H-1 terminology of Stark *et al.*²

The recombinant was discovered among progeny of the backcross HO♂ × (DA × HO)F₁♀. The progeny were screened with three tests defining the segregating AgB4 allele:

- (1) For the presence or absence of AgB4 alloantigens on the surface of their blood lymphocytes recognised by the alloantiserum (AO × HO)F₁ anti-DA in a one-stage cytotoxic test assayed by Trypan blue exclusion.
- (2) For the ability or inability of their blood lymphocytes to stimulate HO thoracic duct lymphocytes (TDL) in a mixed lymphocyte interaction (MLI).
- (3) For the ability or inability of their blood lymphocytes to respond in an MLI against (DA × HO)F₁ spleen cells.

The three tests gave highly concordant results on HO × (DA × HO)F₁ backcross progeny, indicating that all three tests defined a single genetic system (Table 1). One rat showed aberrant behaviour in that her cells, although killed by (AO × HO)F₁ anti-DA serum, responded to (DA × HO)F₁ spleen cells in MLI and did not noticeably stimulate HO TDL. This rat was backcrossed to the HO strain. Of her two litters, 8 out of 21 rats carried serological determinants recognised by (AO × HO)F₁ anti-DA serum, eliminating the slight possibility that the positive result in the serological test of the mother was caused by a rare co-segregation of several minor antigens recognised by the serum. The reactions in the three screening assays of one serologically positive member of these litters are shown in Table 1. The putative recombinant haplotype and tissue will provisionally be called 1R.

The next experiments showed that antigenic products of both the regions of AgB recombined in the 1R chromosome were serologically recognisable, but differed markedly in tissue distribution. First, (AO × HO)F₁ anti-DA serum was absorbed with 1R lymphoid tissue. DA lymphoid tissue absorbed all activity from the serum, whereas even a massive amount of 1R tissue absorbed the serum partly but not completely (Fig. 1a). As all the detectable activity of (AO × HO)F₁ anti-DA was directed against AgB4 (Table 1), the absorption result implies that the 1R haplotype carries some serologically detectable AgB4 alloantigens, but lacks others.

Suitable genetic combinations were therefore chosen to raise separate alloantisera against the two adjacent portions of AgB4. (1R × HO) heterozygotes were immunised against DA, and (AO × HO)F₁ rats against (1R × HO) heterozygous lymphoid tissue. In a lymphocytotoxic test the (AO × HO)F₁ anti-1R serum killed all lymphocytes in blood, lymph nodes and

Table 1 (AO × HO) anti-DA serum detects antigens of the MHC. Tests on a panel of HO × (DA × HO)F₁ backcross progeny

Rat no.	(AO × HO) anti-DA(1/192) % blood lymphocytes killed	Complement control % dead	MLI Response to (DA × HO)F ₁ spleen	MLI stimulation of HO
1	4.1	4.4	4.32 (0.04) (A)	2.40 (0.23) (E)
2	8.1	6.3	4.10 (0.11) (A)	3.09 (0.16) (D)
3	8.7	9.8	4.30 (0.02) (A)	3.38 (0.20) (D)
4	97.1	4.4	2.67 (0.15) (A)	4.66 (0.03) (D)
6	97.6	7.2	2.62 (0.23) (B)	4.58 (0.09) (D)
25	94.9	7.4	2.58 (0.25) (B)	4.59 (0.03) (E)
27	96.9	5.9	2.68 (0.30) (B)	4.58 (0.06) (E)
31	96.3	5.0	2.91 (0.08) (C)	4.49 (0.21) (E)
32	4.3	4.0	4.44 (0.03) (C)	2.50 (0.07) (F)
34	96.0	4.6	2.76 (0.14) (C)	4.43 (0.06) (F)
38	94.3	4.2	2.97 (0.10) (C)	4.19 (0.08) (F)
1R heterozygote*	82.0	3.4	4.44 (0.05) (C)	2.21 (0.10) (F)
	Experiment	Response control	Syngeneic control	
	A	4.25 (0.07)	2.75 (0.08)	
	B	4.39 (0.06)	2.48 (0.08)	
	C	4.11 (0.11)	3.05 (0.16)	
	D	4.69 (0.03)	3.38 (0.11)	
	E	4.65 (0.03)	3.22 (0.16)	
	F	4.22 (0.08)	2.17 (0.11)	

Lymphocytotoxic tests were performed in a total volume of 75 µl, containing 5×10^4 target cells and guinea pig serum at a final concentration of 8–11% (v/v). The suspension medium was Dulbecco's A and B salts supplemented with 2% foetal calf serum. Percentage cell lysis was determined by Trypan blue exclusion, more than 270 cells being counted in each test. The complement control lacked any antiserum. Mixed lymphocyte cultures contained 2×10^5 responding cells and 3×10^5 stimulating spleen cells (irradiated with 950 rad of 16 MeV X rays), in RPMI medium supplemented with 5% rat serum and 2.5×10^{-5} M 2-mercaptoethanol in a total volume of 0.2 ml in round-bottomed microtitre plates. Methyltritiated thymidine (16 Ci mmol^{-1} ; 0.1 µCi in 10 µl) was added at 90–100 h and cells were collected about 16 h later. For MLI responses to (DA × HO)F₁ spleen, responding lymphocyte populations were prepared from cardiac blood by Ficoll-Isopaque separation³. Spleen cells were used to stimulate HO thoracic duct lymphocytes. Mixed lymphocyte responses are expressed as the log₁₀ geometric mean c.p.m. of at least three replicate cultures. The standard deviation from the log₁₀ geometric mean c.p.m. in replicate cultures is in the first parentheses after the response. In the second parentheses, A–F are names for the experiments for which the response controls and syngeneic controls are given below the main body of the table; in A–C the response control is the response of HO blood lymphocytes to (DA × HO)F₁ spleen; in D–F the response control is the response of HO thoracic duct lymphocytes to (DA × HO)F₁ spleen.

*The results for 1R heterozygote were obtained with one of the original recombinant's second litter by an HO male.

thoracic duct of rats carrying AgB4 (Fig. 1b). It was also strongly and specifically lytic for red cells of such rats. The target antigen(s) on both red cells and lymphocytes is AgB-linked as established through five backcross generations of both AgB4 and the 1R haplotype on to the HO background. The target(s) for this antiserum seems therefore to be analogous to the mouse H-2K and H-2D molecules, which are also expressed on both lymphocytes and erythrocytes^{4,5}.

The (1R × HO) anti-DA serum was considerably different. Although of high titre, it killed only 30–40% of normal lymphocytes (Fig. 1b), and was not detectably haemolytic. Furthermore, it seemed that the target for this antiserum was restricted to B lymphocytes. Using lymphocytes collected from the thoracic duct of a thymectomised, irradiated, bone-marrow reconstituted 'B' rat⁶ of the DA strain it was found that > 90% of 'B' TDL were killed by (1R × HO) anti-DA serum while only 35–40% of normal TDL were killed in the same test (Fig. 1b). Similarly, the (AO × HO)F₁ anti-DA serum absorbed exhaustively with 1R lymphoid tissue killed about 90% of 'B'

TDL compared with 35–40% of normal TDL (Fig. 1a). (1R × HO) anti-DA serum is thus analogous to some mouse anti-Ia sera in the apparent absence of the target antigens from erythrocytes and from T cells^{7,8}. It was necessary to demonstrate that the targets of (1R × HO) anti-DA and (AO × HO)F₁ anti-1R are controlled by linked portions of AgB. For this purpose we returned to the progeny of the HO × (DA × HO)F₁ backcross amongst which the putative recombinant was found. The two antisera were tested on blood lymphocytes of a panel of these rats whose AgB status had already been established. There was complete positive correlation of the activity of the two antisera and both detected antigens of AgB4 (Table 2). Thus (1R × HO) anti-DA and (AO × HO)F₁ anti-1R are directed at antigens controlled by different segments of a single genetic system.

The recombinant haplotype, 1R, has inherited its major serological antigens (analogous to the mouse K and D molecules) from the AgB4 of DA, and lacks antigen(s) of AgB4 seemingly analogous to mouse Ia antigens. It has also inherited its MLI phenotype principally from AgB5, the (1R × HO)

Table 2 Genetic linkage test for the targets of (AO × HO)F₁ anti-1R and (1R × HO) anti-DA using a panel of HO × (DA × HO)F₁ backcross progeny

Rat	AgB4	Complement control (% cells dead)	% cells killed by (1R × HO) anti-DA		% cells killed by (AO × HO) anti-1R	
(DA × HO)F ₁	+	6.1	1/6	1/24	1/6	1/192
26	+	7.8	ND	23.0	ND	92.1
30	+	4.2	ND	27.7	ND	96.9
42	+	8.0	ND	28.2	ND	94.6
44	+	11.4	ND	23.0	ND	94.4
45	+	5.5	ND	25.2	ND	93.5
46	+	4.4	ND	20.6	ND	93.6
33	—	7.5	ND	20.6	ND	95.3
35	—	11.8	7.3	6.7	5.6	4.9
36	—	3.7	9.9	7.1	8.6	11.1
37	—	7.3	3.5	2.3	3.2	5.1
43	—	5.0	5.5	7.5	6.5	6.9
			6.9	2.5	5.5	6.6

The AgB status of each of these animals was determined by both serological and MLI tests, as described in Table 1. Lymphocytotoxic scores are based on a sample of > 300 cells. ND, not determined.

Table 3 MLI responses of HO and (1R×HO) heterozygote TDL to (DA×HO)_F₁ spleen

Responder TDL (2 × 10 ⁵)	Stimulator spleen cells (3 × 10 ⁶)	Response
HO	HO (syngeneic control)	2.58 (0.02)
	(1R × HO) heterozygote*	2.47 (0.08)
	(DA × HO) _F ₁	4.73 (0.03)
(1R × HO) heterozygote*	HO	2.66 (0.03)
	(1R × HO) heterozygote (syngeneic control)	2.46 (0.05)
	(DA × HO) _F ₁	4.72 (0.04)

MLI Response data is presented as in Table 1.

*The (1R×HO) heterozygotes used in this experiment were offspring of the original recombinant, previously shown to be carrying DA serological determinants by positive reaction with (AO×HO)_F₁ anti-DA serum.

heterozygote behaving just like HO in this experiment (Tables 1 and 3). Five backcrosses of the 1R haplotype on to the HO background have now been completed.

The analysis of this recombinant demonstrates that the region of AgB controlling the MLI and Ia-like antigens can be recombined by a single cross over from the region controlling cell-surface alloantigens of wide tissue distribution analogous to the K and D molecules of the mouse. The properties of the

recombinants described by Williams and Moore⁹, of the KGH rat strain investigated by Gill and co-workers^{10,11} and of the AgB haplotype derived from a wild rat by Gasser¹² support this view. Although the existence of more than one K/D-like locus has not been demonstrated in the rat, it seems that the linear order of genes in AgB is not like that in the H-2 of the mouse, where the D and K loci flank the region controlling the MLI and Ia antigens. The rat may fit better the model established for the human MHC¹². Indeed, the present study further emphasises the conclusion derived from other more distant mammalian species including man¹³, that the gene order in the mouse MHC is exceptional, presumably as a result of a chromosome rearrangement which can now be placed as more recent than the divergence of the mouse and the rat from their common ancestor. We consider, however, that our conclusion on the structure of the rat MHC is still preliminary. Confirmation from other more informative laboratory recombinants is awaited, because special circumstances such as the undetected sharing of a second K/D-like chromosome region by the AgB4 and AgB5 haplotypes could invalidate the conclusion.

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Note added in proof: A recombination between AgB4 and AgB2 (H-1^a and H-1^w) has recently been found¹⁵. This recombinant seems to have similar properties to that we have described.

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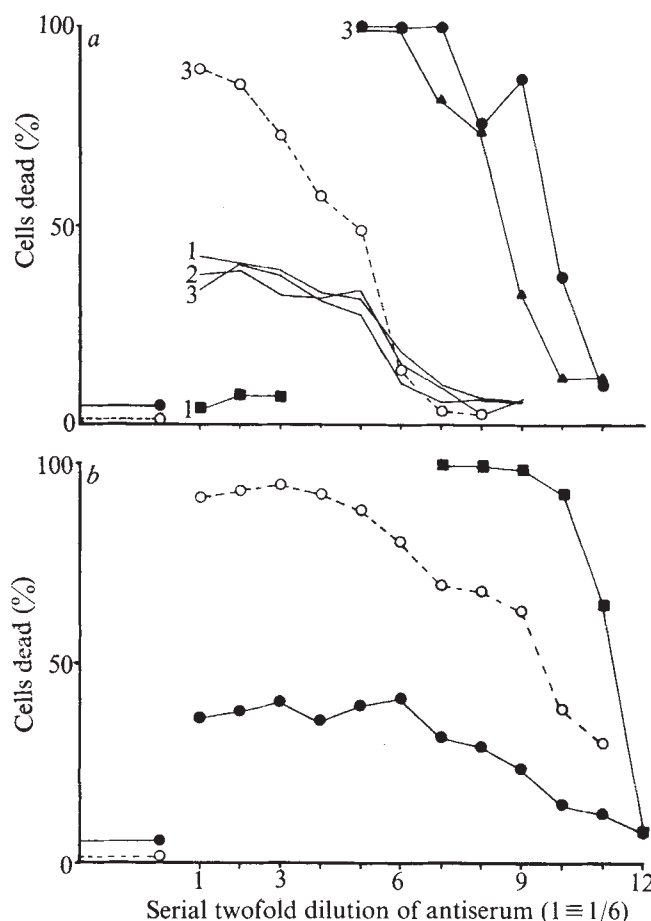


Fig. 1 Titrations of cytotoxic antisera against DA lymphoid cells (methods as in Table 1). *a*, (AO×HO)_F₁ anti-DA absorbed with 1R lymphoid tissue. (●—●), (AO×HO) anti-DA tested on DA lymph node cells; (—), (AO×HO) anti-DA absorbed with 1R tested on DA lymph node cells. 1, 2, 3 are successive stages in the absorption. At each stage more than 700×10⁶ lymphocytes were used per ml of antiserum; (○—○), (AO×HO) anti-DA absorbed with 1R at stage 3 of absorption, tested on 'B' DA TDL; (■—■), (AO×HO) anti-DA absorbed with DA at stage 1; (▲—▲), control absorption with HO at stage 3; (—●—), complement control for DA lymph node cells; (—○—○), complement control for 'B' DA TDL. *b*, Recombinant antisera tested on DA TDL. (■—■), (AO×HO)_F₁ anti-1R on normal DA TDL; (●—●), (1R×HO) anti-DA on normal DA TDL; (○—○), (1R×HO) anti-DA on 'B' DA TDL. Complement controls as in (*a*).

A surface antigenic marker for rat Schwann cells

THE use of cell-surface antigenic markers to identify and separate distinct subpopulations of lymphocytes has revolutionised immunology in recent years. It seems likely that a similar approach will be fruitful in other areas of biology. For example, recent advances in maintaining and studying cells of the nervous system *in vitro* have created a pressing need for cell-type-specific markers. These would allow unequivocal identification and

possible purification of the many different types of neural cells so that their interactions can be studied in culture. Here we describe a cell-surface antigen, defined by an antiserum raised in mice against a rat neural tumour cell line¹ which is present on Schwann cells in dissociated cell culture of neonatal rat sciatic nerve, but not on fibroblasts in the same culture. Another cell-surface antigen, Thy-1.1 (formerly called θ AKR)² is expressed by the fibroblasts³ and not the Schwann cells, making it possible to mark both cell types simultaneously in these cultures by using antibodies conjugated to two different fluorochromes.

The mouse antiserum was raised against a cell line (33B) derived from an ethylnitrosourea-induced tumour arising in the spinal cord and nerve roots of a Wistar-Furth (W/Fu) rat¹. After extensive adsorption with rat liver, spleen and thymus, the original serum defined two surface antigens detected by cytotoxicity testing: (1) a 'common antigen' present in rat brain, embryonic tissues and most tumours or cell lines from the rat nervous system (including Schwannomas, gliomas and neuroblastomas) and (2) a 'restricted antigen' present on a minority of nervous system tumours and cell lines and not detectable in normal or embryonic rat tissues¹. For the studies described here, we used an antiserum, raised in A/strong mice⁴, with cytotoxic activity at least 80% specific for the common antigen. After the usual tissue adsorptions, it was further adsorbed twice with half its volume of packed cells obtained from primary muscle cultures of neonatal W/Fu rats. The

at 100,000*g* for 30 min before use, and for double labelling experiments the G-anti-MIg-Rd was adsorbed with rabbit IgG and the G anti-RIg-F1 with mouse IgG. The rabbit and mouse IgG were coupled to Sepharose 6B.

Sciatic nerves were removed aseptically from neonatal W/Fu rats, digested with trypsin and collagenase, and dissociated by passage through a No. 23 hypodermic needle. The cells were grown on collagen-coated glass coverslips in Linbro dishes (Flow Labs.) in Dulbecco's modified Eagle's medium, containing penicillin (100 IU per ml), streptomycin (100 μ g ml⁻¹), 10% heat inactivated foetal calf serum and 2% chick embryo extract. A full account of this procedure will be published elsewhere. After 3–5 d of culture, the cells on the coverslips were incubated in anti-Ran-1 or anti-Thy-1 antisera for 30 min at room temperature, washed well and incubated with the appropriate fluorescent anti-Ig antibodies for a further 30 min at room temperature. After washing, the cells were fixed in 5% glacial acetic acid in 95% ethanol at 4 °C for 10 min, mounted in Elvanol on a glass slide and examined using a Zeiss fluorescence microscope equipped with phase contrast and incidence fluorescence optics.

In 3-d-old cultures, the majority of cells had a bipolar morphology characteristic of Schwann cells in culture⁵ with a tendency to line up end-to-end; by 5 d the long processes of these cells tended to form interconnected networks. The other cells in the culture had the appearance of fibroblasts; initially sparse, they tended to proliferate and form a confluent underlayer by 7 d.

Table 1 Indirect immunofluorescence labelling of Schwann cells and fibroblasts in rat sciatic nerve cultures with anti-Ran-1 and anti-Thy-1 antiserum

Antiserum	Adsorbed with	Labelling of	
		Schwann cells	Fibroblasts
Mouse anti-Thy-1.1	—	0	+
Mouse anti-Thy-1.1	CBA (Thy-1.2) brain	0	+
Mouse anti-Thy-1.1	AKR (Thy-1.1) brain	0	0
Rabbit anti-Thy-1	—	0	+
Rabbit anti-Thy-1	Rat (W/Fu) thymocytes	0	0
Mouse anti-Ran-1	—	+	0
Mouse anti-Ran-1	Rat neural tumour line 33B	0	0
Mouse anti-Ran-1	Rat neural tumour line 21A	0	0
Mouse anti-Ran-1	Rat neural tumour line B28	+	0
Mouse anti-Ran-1	Rat foetus	0	0
Mouse anti-Ran-1	Rat liver	+	0

Cells were labelled with mouse anti-Thy-1.1 (1:5) or mouse anti-Ran-1 (1:50) and then G anti-MIg-Rd (1:20), or with rabbit anti-Thy-1 (1:20) and mouse anti-Ran-1 (1:50) simultaneously, followed by F(ab)₂ G anti-RIg-F1 (1:10) and G anti-MIg-Rd (1:20) applied simultaneously.

antigen detected by immunofluorescence on Schwann cells has a similar distribution on tumour cell lines to that of the common antigen (see below). The exact identity of the Schwann cell antigen and the common antigen is, however, not yet completely established. We shall therefore refer to the antigen detected on Schwann cells as rat neural antigen-1 (Ran-1).

Anti-Thy-1.1 alloantiserum was raised in CBA mice against AKR thymocytes as described before⁵; in indirect immunofluorescence assays it labelled AKR and rat thymocytes but not CBA thymocytes, and all of this activity could be adsorbed by brain from AKR but not from CBA mice. Rabbit anti-Thy-1 antiserum was raised by immunising rabbits with rat brain glycoproteins (obtained after lentil lectin affinity chromatography) and boosting with purified Thy-1 5 months later⁶. It was adsorbed twice with 50% (v/v) packed rat spleen cells and twice with 50% (v/v) packed neonatal rat brain homogenate (both of which are known^{7,8} to contain relatively little Thy-1). For indirect immunofluorescence assays, rhodamine-conjugated goat anti-mouse IgG (G anti-MIg-Rd) and fluorescein-conjugated goat anti-rabbit IgG (G anti-RIg-F1) antibodies Nordic batch no. 2-773 and 27-175, respectively) were used. In most experiments, the F(ab)₂ fragments of these conjugates were used. They were prepared by dialysing the undigested reagents in 0.1 M acetate pH 4.5 and incubating them with 2% (w/w) pepsin (Sigma) for 12 h at 37 °C, dialysing in phosphate buffered saline and passing them through an XM50 Amicon filter to remove the Fc fragments. All antisera were centrifuged

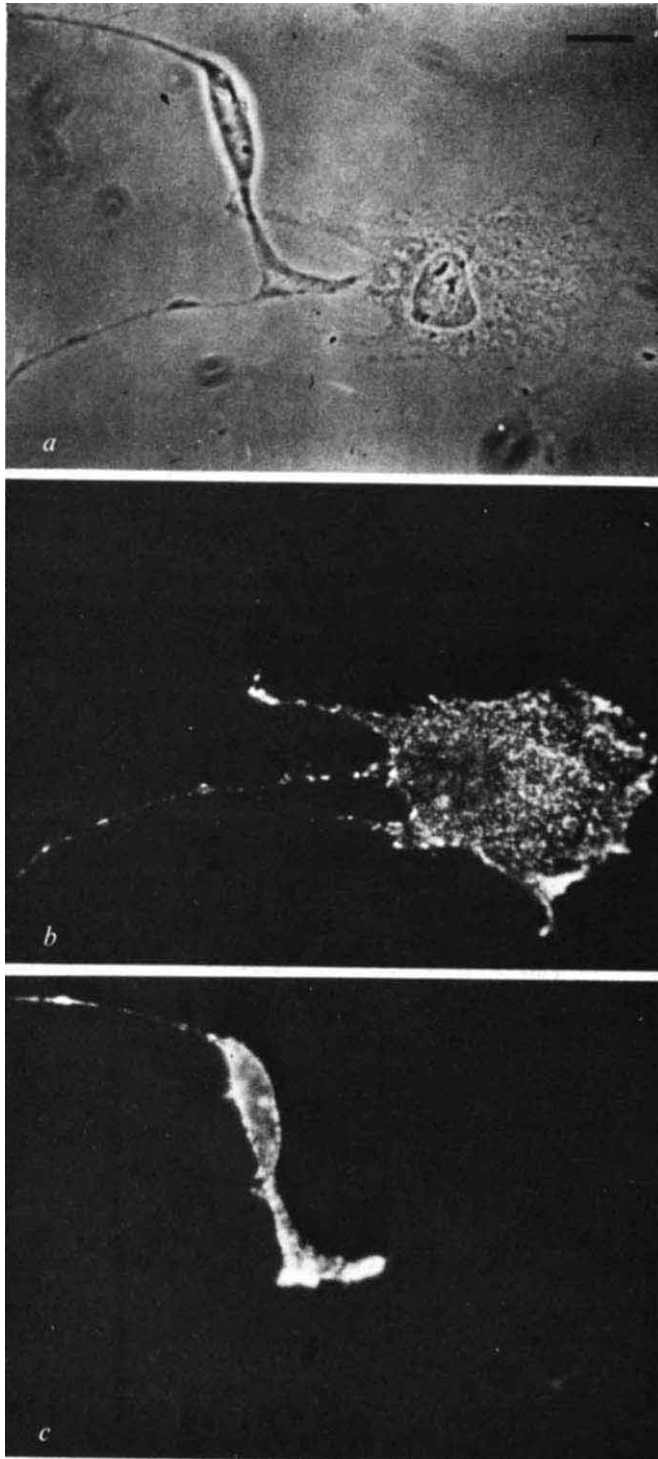
Every one of the characteristic bipolar Schwann cells was brightly labelled by the anti-Ran-1 serum and did not label with the mouse or rabbit anti-Thy-1 antiserum. On the other hand, all of the typical fibroblasts were labelled by both of the anti-Thy-1 sera, and not by the anti-Ran-1 serum. In all cultures, there were cells with an intermediate morphology, which stained as typical Schwann cells.

The controls for the specificity of the immunofluorescence labelling are listed in Table 1. The labelling activity of the mouse anti-Thy-1.1 antiserum was removed by adsorption with AKR but not CBA brain, confirming the Thy-1.1 specificity of the antiserum. The labelling activity of the rabbit anti-Thy-1 serum was removed by adsorption with rat thymocytes. The labelling of Schwann cells by anti-Ran-1 could be removed by adsorption with cell line 33B, which has both the common and the restricted antigen, or by line 21A, which has the common antigen but lacks the restricted antigen. Line B28 (derived by D. Schubert at the Salk Institute from a brain tumour) lacks both antigens and does not adsorb the Schwann cell labelling. Adsorptions with rat brain, which contains less of the antigen than the tumour lines or rat foetus, have thus far been inconclusive. With this reservation, these data suggest that the immunofluorescent labelling of Schwann cells is detecting the common antigen.

To prove that the anti-Ran-1 and anti-Thy-1 antisera were not labelling the same cells, cultures were exposed to rabbit anti-Thy-1 and mouse anti-Ran-1 antisera simultaneously,

washed and then incubated in G-anti-R1g-F1 and G anti-M1g-Rd simultaneously; after washing, the cells were examined for fluorescein and rhodamine fluorescence. In most cultures, all cells labelled with one or other fluorochrome but not both (Fig. 1; also see cover). In an occasional culture, a small proportion (<5%) of the cells were labelled with both fluorochromes; these cells had a distinctive round morphology and

Fig. 1 Indirect immunofluorescence using antibodies against Thy-1 and Ran-1. Cells from sciatic nerve cultures were stained with a mixture of rabbit anti-Thy-1 and mouse anti-Ran-1, followed by a second incubation with (Fab)₂ G-anti-R1g-fluorescein and F(ab)₂ G anti-M1g-rhodamine, as described in the text. A pair of cells was photographed with phase optics (a) or ultraviolet illumination and filters to detect the fluorescein (anti-Thy-1)-labelled fibroblast (b), or filters to detect the rhodamine (anti-Ran-1)-labelled Schwann cell (c) Scale bar, 10 μ m.



were also doubly labelled when normal mouse and rabbit sera were used in the first incubation in place of the antisera. They could be damaged cells or macrophages.

In preliminary experiments, the double labelling method has been used to investigate cell division in the two populations. Two-day cultures were incubated with ³H-thymidine for 24 h, stained with the two fluorochromes, and coated with emulsion for conventional light microscope autoradiography. Whereas approximately 90% of the Thy-1 positive cells had labelled nuclei, only 20% of the Ran-1 positive cells had them. The latter result is comparable with that obtained in neonatal mouse sciatic nerve *in vivo*¹⁰. This method should be useful in the search for possible Schwann cell mitogens.

We conclude from these studies that in dissociated cell cultures of newborn rat sciatic nerve it is possible to distinguish Schwann cells from fibroblasts by means of surface antigenic markers: the Schwann cells express the previously described¹ Ran-1 but not detectable amounts of Thy-1, while fibroblasts do not express detectable amounts of Ran-1 but do express Thy-1. Although the Thy-1 positive cells had a typical fibroblastic morphology, it is possible that this category may include other cell types. Thus, endothelial cells are present in peripheral nerve^{10,11} and they have not been typed for the presence of Thy-1. The identity of the Ran-1 positive cells with intermediate morphology is not seriously in doubt. Studies in culture with time-lapse cinematography¹² have shown that bipolar Schwann cells may undergo a morphological transition to a fibroblast-like form. Although the detailed distribution of Ran-1 in the nervous system is not known, it is not detectable by immunofluorescence on any major class of cells in dissociated cultures of cerebellum or cerebral hemispheres (unpublished observations of K.L.F.); thus it is not a general glial marker. It will be of interest to compare the characteristics of this antigen and the recently described surface antigen on human Schwann cells¹³.

Since the fibroblasts³, but not Schwann cells, can be killed by anti-Thy-1 and complement (unpublished observations) it should be possible to remove the fibroblasts from sciatic nerve cultures; alternatively, the Schwann cells could be positively selected by the use of a fluorescence activated cell sorter¹⁴. In either case, the availability of surface markers for both cell types should prove useful in studies of myelination *in vitro*, and of other interactions with nerve¹⁵ and muscle¹⁶. Moreover, the availability of one neural cell marker should facilitate the search for others.

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Developmentally regulated lectin in neonatal rat brain

WE report the identification of a developmentally regulated agglutinin of erythrocytes present in neonatal rat brain. Specific inhibition by a number of saccharides with a galactose configuration suggest that multivalent carbohydrate-binding proteins, 'endogenous brain lectins', may be responsible for the observed agglutination activity. We are presently investigating the functional role of this lectin-like activity in brain, since molecules with similar carbohydrate-binding properties seem likely to mediate intercellular recognition and adhesion in certain lower eukaryotic organisms.

The ability of cells to recognise other cells and specifically adhere to them is a central problem in developmental neurobiology and the subject of intense investigation. Moscona¹, more than a decade ago, proposed that specific macromolecules at the cell surface are responsible for specific adhesion. Specific cell recognition and adhesion could then be achieved by a cell-surface macromolecule, a carbohydrate-binding protein² or an enzyme³ with a particular recognition specificity, and a carbohydrate-containing receptor, one or both of which is under a temporal metabolic regulation. The lectin hypothesis of cell recognition and adhesion² has gained support in recent studies⁴⁻⁸ with the cellular slime moulds (for review, see ref. 9). Developmentally regulated carbohydrate-binding proteins, "discoidin"⁴ and "pallidin"^{7,8} accumulate on the cell surface of *Dictyostelium discoideum* and *Polysphondylium pallidum* as these species differentiate from a vegetative single cell state to a cohesive aggregation-competent state, and substantial evidence^{2,4-8} indicates that these slime mould lectins may mediate intercellular recognition and adhesion by interacting with specific cell-surface receptors⁷. Teichberg *et al.*¹⁰ reported the presence of a β -D-galactoside-binding protein in *Electrophorus electricus* and in a number of mammalian tissues and tissue culture cells, and Nowak *et al.*¹¹, and Gartner and Podleski¹², have studied a β -D-galactoside binding protein in embryonic chick muscle and myogenic cell lines, a protein, purified and characterised by Den and Malinzak¹³.

The functional components of intercellular recognition and adhesion are generally thought to be glycoproteins¹⁴ and in brain tissue in particular, the synaptic specialisation is enriched by carbohydrate-containing macromolecules which may function in synaptogenesis^{14,15}, in conjunction with carbohydrate-binding molecules of appropriate specificity.

Soluble extracts of developing neonatal rat brain contain a 'lectin-like' agglutination activity which is measured with trypsinised glutaraldehyde-fixed rabbit erythrocytes. In brain extracts prepared from 1-30-d-old rats (Fig. 1) a marked increase in agglutination activity was observed from day 1-10, followed by a decline to undetectable levels between 10 and 21 d. Only trace amounts of activity were found in adult animals. The time course seen in Fig. 1 was reproduced with different sets of brain extracts, and different preparations of rabbit erythrocytes, although the absolute value of the titre varied somewhat with the particular preparation of erythrocytes. Variable agglutinability of erythrocyte preparations could result from individual donor differences or from minor variations in the conditions of trypsinisation or glutaraldehyde fixation. Specific agglutination activity—that is, agglutination activity per mg protein—parallels the curve seen in Fig. 1. The possibility that the observed time course was a function of changes in the extracting reagent (DES) or in the agglutinability of the erythrocyte preparation over the time course of the study was eliminated by preparing extracts at the individual time points, freezing and assaying them together on the same day, or by simultaneously preparing extracts of animals at various ages and assaying on the same day. Mixing experiments in which day 1 and 10 extracts and day 10 and 30 extracts were mixed, incubated and assayed, gave intermediate values

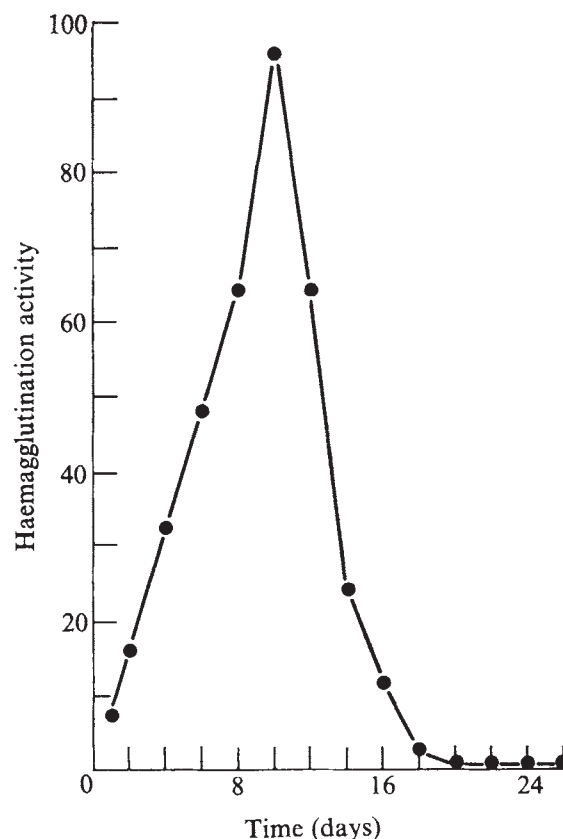


Fig. 1 Sprague-Dawley pups from litters normalised to eight pups per mother at birth, to ensure that all had equal and sufficient nutrition, were killed by decapitation. Brains were homogenised at 4 °C in 4 volumes of freshly prepared 2 mM dithiothreitol, 2 mM EDTA, 0.15 M NaCl, abbreviated DES, in a Potter-Elvehjem tissue homogeniser. Dithiothreitol at 10 mM did not improve the extractability of activity but served to stabilise the activity extracted. The homogenate was centrifuged 60 min at 40,000 r.p.m. and the supernatant assayed for agglutination activity using trypsinised glutaraldehyde-fixed rabbit erythrocytes. Erythrocytes for agglutination assays were prepared from rabbit blood (Colorado Serum Labs) collected in Alsever's solution. Cells were washed 4 times with 5 volumes saline. Erythrocytes were suspended 4% (v/v) in 0.1 M sodium phosphate-0.05 M NaCl, pH 7.4, and trypsin from bovine pancreas (Sigma) 0.1 mg ml⁻¹, was added, and the suspension incubated at 37 °C for 60 min. Cells were then washed 5 times with 10 volumes saline. Washed erythrocytes were suspended in 5 volumes 0.075 M Na, K phosphate-0.075 M NaCl, pH 7.2 (PBS 7.2), containing 1% glutaraldehyde (Sigma, grade I), and incubated with shaking for 1 h at room temperature. The cross-linking reaction was terminated by addition of 5 volumes 0.1 M glycine in PBS 7.2 at 4 °C. Erythrocytes were sedimented and washed 3 times with 5 volumes 0.1 M glycine in PBS 7.2. Finally, cells were washed 4 times in 5 volumes cold PBS 7.2. Agglutination activity in brain extracts was measured with trypsinised glutaraldehyde-fixed rabbit erythrocytes as follows. A twofold dilution series of brain extract was made in DES. Either 25 μ l PBS 7.2 or 25 μ l an appropriate hapten inhibitor in PBS 7.2 was added to wells of a microtitre V plate (Cooke Engineering). Aliquots (25 μ l), of the serial dilutions of extract were added to wells followed by 25 μ l 1% solution bovine serum albumin in 0.15 M NaCl. After gentle mixing, 25 μ l of a 2.5% suspension of erythrocytes in PBS 7.2 was added to wells. Plates were shaken vigorously, covered with transparent tape, and left at room temperature for 90 min before agglutination patterns were evaluated. The agglutination endpoint was taken as the first dilution of extract at which erythrocytes settled into a clearly circumscribed dot at the bottom of the well. Agglutination activity is defined as the reciprocal of the endpoint dilution.

suggesting that enzymatic modification in crude soluble extracts was not responsible for the ascending or descending portions of the time course. No agglutination was observed when serum from 10-d-old rats was tested for this activity.

A number of other types of erythrocytes were tested for their

ability to calibrate agglutination activity. Fresh rabbit erythrocytes were not agglutinable, nor did trypsinisation of fresh erythrocytes render them agglutinable. Fixation with glutaraldehyde or formalin was required to sensitise the rabbit cells to agglutination, whereas trypsinisation before fixation gave 50% higher titres suggesting that such treatment, although insufficient in itself, does increase the agglutinability of fixed cells. Trypsinised formalin-fixed rabbit erythrocytes and trypsinised glutaraldehyde-fixed or formalin-fixed sheep erythrocytes were four times less sensitive to agglutination than the trypsinised glutaraldehyde-fixed rabbit erythrocytes, whereas formalin-treated human type O erythrocytes were eleven times less sensitive.

Agglutination activity of 10-d-old rats was specifically inhibited by a number of monosaccharides and disaccharides with a galactose configuration, in addition to hexosamines, mannose, and a brain glycopeptide fraction¹⁶, as seen in Table 1. This non-dialysable glycopeptide fraction was found to be the most potent inhibitor of agglutination, being nearly 150 times greater on a molar basis than the most effective monosaccharide inhibitor, galactosamine. The glycopeptide fraction, with an average molecular weight of 8,000, was prepared from lipid-extracted whole brain by extensive proteolytic digestion, followed by quantitative precipitation of contaminating glycosaminoglycans with cetylpyridinium chloride¹⁶. The potent inhibition by hexosamines is interesting in view of the report by Yamada *et al.*¹⁷, that the CSP protein or LETS protein, an agglutinin of formalin fixed erythrocytes, which has been identified as a surface antigen in cultured glial cells¹⁸, was also inhibited by hexosamines. Careful re-examination of the hexosamine inhibition in brain extracts by measuring hexosamine inhibition at various amino sugar concentrations in parallel with equimolar concentrations of NaCl, suggested that the inhibition at high concentrations was predominantly a charge effect, whereas studies at lower hexosamine concentrations revealed a galactosamine-specific inhibition. Agglutination by the brain extract is probably not due to LETS protein since EDTA or EGTA, potent inhibitors of the LETS mediated agglutination¹⁷, were not potent inhibitors of the brain activity. Nevertheless, we cannot absolutely rule out the possibility that the brain activity, in part, is LETS mediated, since a different cell type was used to test agglutination by purified LETS protein. An accurate comparison to the hapten inhibition specificity of the LETS protein, awaits purification of the brain agglutinin.

Although the sugar concentrations required to inhibit agglutination by brain extracts are relatively high, unlike those of many lectins derived from plant sources, a number of lectins do require concentrations in this range. The carbohydrate specificity, although generally galactose specific, is not absolute suggesting that several carbohydrate-binding proteins with

distinct specificities may be present in crude extracts from 10-d-old rats.

Extracts containing agglutination activity were subjected to a variety of enzymatic and chemical treatments described in Table 2. The carbohydrate-binding protein was extremely sensitive to trypsin and chymotrypsin and somewhat less sensitive to neuraminidase. No decrease in activity was observed after incubation with a variety of other enzymes. Agglutination activity was quantitatively inactivated by heating at 100°C for 5 min. Mild periodate oxidation in controlled conditions¹⁹ also quantitatively destroyed the agglutination activity. The effects of trypsin, chymotrypsin, neuraminidase and periodate oxidation suggests that both protein and carbohydrate components are in some way required for agglutination.

Table 2 Enzymatic and chemical characterisation of carbohydrate-binding activity in developing rat brain

Enzymatic or chemical treatment	Concentration ($\mu\text{g ml}^{-1}$)	Decrease in activity (%)
Trypsin	0.1	25
	1	100
	10	100
	100	100
Chymotrypsin	0.1	0
	1	100
	10	100
	100	100
Neuraminidase	200	75
	100	50
β -glucosidase	200	0
Collagenase	200	0
Hyaluronidase	200	0
Phospholipase D	200	0
Ribonuclease	200	0
Deoxyribonuclease	200	0
Heat (5 min, 100°C)	—	100
Sodium periodate (1 h, 4°C)	0.012 M	100

Rat brain extract from 10-d-old pups was incubated with a series of enzymes at the specified final concentrations for 1 h at 37°C. Controls without enzyme and without extract were incubated and assayed for agglutination activity in parallel experiments. Trypsin sensitivity was also demonstrated when the action of trypsin was terminated after incubation by addition of excess trypsin soybean inhibitor before assay, or when a Sepharose-trypsin conjugate (Sigma) was used. Also a decrease in agglutination could be demonstrated in experiments using a Sepharose-neuraminidase conjugate (Sigma), although this effect may be due in part to the action of protease contaminants. Mild treatment with sodium periodate in controlled conditions quantitatively inhibited agglutination activity. Incubation conditions used were designed for the selective oxidation of terminal sialic acid residues¹⁹.

The heat lability, enzyme studies, and the hapten inhibition specificity suggest that the observed agglutination is not a result of the adventitious association of glycosaminoglycans or nucleic acids with erythrocytes. The activity we have identified in rat brain does not seem to be related to an agglutinin of formalin treated chick erythrocytes which accumulates in chick brain during embryonic development²⁰. Agglutination produced by this extremely large proteoglycan-like complex cannot be inhibited by specific saccharides and its activity is insensitive to boiling.

The peak of agglutination activity (Fig. 1) is coincident with the time course of maximum synapse formation in developing rat brain cortex²¹. It is also interesting that the agglutination activity seems to parallel the temporal course of maximum *in vitro* cohesiveness of homotypic interactions of disassociated neuronal cells^{22,23}. These parallels are at best suggestive, and further experiments are required to implicate functionally this agglutination activity in the specific cellular interactions required for synaptogenesis and neuronal development.

We thank Dr S. D. Rosen for discussions and Dr Rosen and

Table 1 Glycopeptide and sugar effects on agglutination of erythrocytes by brain extracts

Sugar concentrations (mM) that inhibit agglutination by 50%			
Brain glycopeptides	0.1	<i>N</i> -acetylglucosamine	> 125
Galactosamine	15	<i>N</i> -acetylmannosamine	> 125
α -lactose	30	Glucose	> 125
Thiodigalactoside	30	L-fucose	> 125
Mannosamine	30	L-rhamnose	> 125
Glucosamine	30	α -methyl-D-galactose	> 125
Mannose	60	α -methyl-D-glucose	> 125
Galactose	60	α -methyl-D-mannose	> 125
<i>N</i> -acetylglactosamine	> 125	2-deoxy-D-glucose	> 125

Brain extract from 10-d-old rats was assayed for agglutination activity as described in Fig. 1 in the presence of a series of concentrations of various sugars in PBS 7.2 to determine the sugar concentration that would inhibit agglutination activity by 50%. In addition, brain glycopeptides, prepared as described by Simpson *et al.*¹⁶, were tested as inhibitors of agglutination. The molarity of the glycopeptide was based on an average molecular weight of 8,000, determined by gel-filtration on Sephadex G-50.

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Effects of glycolipids on *in vitro* development of neuromuscular junction

THE nervous system has a unique distribution of glycolipids¹, which changes markedly during growth of the animals² and in various inherited metabolic disorders³. No physiological roles for glycolipids in neural function are known, however. But, in nonneural cells glycolipids of the cell membrane are essential for cell recognition and interaction⁴. For example, some gangliosides act as receptors for various bacterial toxins⁵ and globoside has been identified as the human P blood type antigen⁶. When administered in the medium, ganglioside can be incorporated into cell membranes and function as toxin receptors^{5,7}. Ganglioside added to the medium competes with membrane receptors by binding to the toxins⁵. We reported

previously^{8,9} that the effects of some drugs and sera on the formation of neuromuscular junction in culture could be evaluated by recording the endplate potential (e.p.p.) from muscle cells combined with the spinal cord. We describe here the effect of glycolipids on the development of neuromuscular junction in culture of skeletal muscle and the spinal cord. Globoside had biphasic effects, an inhibitory effect at the higher concentration and a facilitatory one at the lower concentration or on brief exposure at the beginning of the cultivation. Our evidence suggests that glycolipids are involved in formation of the neuromuscular junction.

Pectoral muscles and spinal cords were obtained from 10–11-d and 5–7-d chick embryos (White Leghorn) respectively. The muscles were dissociated to single cells by incubation in 0.25% trypsin and $2.5\text{--}5 \times 10^5$ cells were plated into a plastic culture dish (35 mm diameter, Falcon). After 2 or 3 d of culturing muscle cells alone, fragments (about 15 pieces) of the spinal cord were added to each dish. The cord fragments were obtained by slicing the whole length of the hemisectioned spinal cord. The culture medium was Eagle's minimum essential medium (MEM, Nissui) supplemented with 10% foetal calf serum, 5% chick serum (both Flow Labs.), 6 mg ml⁻¹ glucose and 0.06 mg ml⁻¹ kanamycin. The cultures were maintained at 37 °C in a humidified atmosphere of 95% air and 5% CO₂. At the time of plating the cord the medium was renewed and glycolipid (Table 1) was added. In some experiments globoside was added according to the schedule described in Table 3. Glycolipids were prepared as before^{10–13}. Galactosylceramide, sulphatide and gangliosides (a mixture of G_{M1}–G_{T1} and G_{M1}) were from bovine brain^{10,11}. Glucosylceramide was isolated from the spleen of a patient with Gaucher's disease, and purified by Florisil and silicic acid column chromatography. Haematoside (N-glycolylneuraminyl-galactosyl-glucosyl-ceramide) and globoside (N-acetylgalactosaminyl-galactosyl-galactosyl-glucosyl-ceramide) were isolated from equine and human erythrocytes respectively^{12,13}. The sugar chain of globoside (N-acetylgalactosaminyl-galactosyl-galactosyl-glucose) was obtained by ozonolysis and mild alkali treatment of globoside^{14,15}, and further purified by charcoal column chromatography. The purity of the oligosaccharide was checked by gas-liquid chromatography¹⁵.

After further 2 d of coculture, the culture dish was mounted on the stage of an inverted phase-contrast microscope and membrane potentials of myotubes (multinucleated muscle cells) were recorded intracellularly with a KCl-filled electrode (resistance 15–20 MΩ). The experiments were usually performed in the fresh MEM without serum and glycolipid at 35–38 °C. The presence of glycolipid in the medium, however, did not interfere with measurement of electrophysiological parameters, and did not alter the results.

A proportion of myotubes, cultured for 2 d in combination with the spinal cord explant, spontaneously generated e.p.ps (ref. 8) (Fig. 1). The e.p.ps varied in amplitude from myotube to myotube (range 1–10 mV) and in frequency (0.5–30 per s). As in the previous studies^{8,9}, the presence of spontaneous e.p.ps was regarded as indicating the formation of a neuro-

Table 1 Spontaneous e.p.ps in myotubes cultured for 2 d with the spinal cord in the presence of glycolipids

Glycolipid	Concentration (mM)	No. of myotubes tested	Myotubes with e.p.ps (%)
None (control)		435	55
Galactosylceramide	0.5	60	63*
Glucosylceramide	0.5	57	56*
Haematoside	0.5	56	41*
Sulphatide	0.05	62	63*
Gangliosides (G _{M1} –G _{T1})	0.5	88	45*
G _{M1}	0.5	57	21†
Globoside	0.5	52	23†

* $P > 0.05$, compared with control by χ^2 test on 2×2 contingency tables.

† $P < 0.01$.

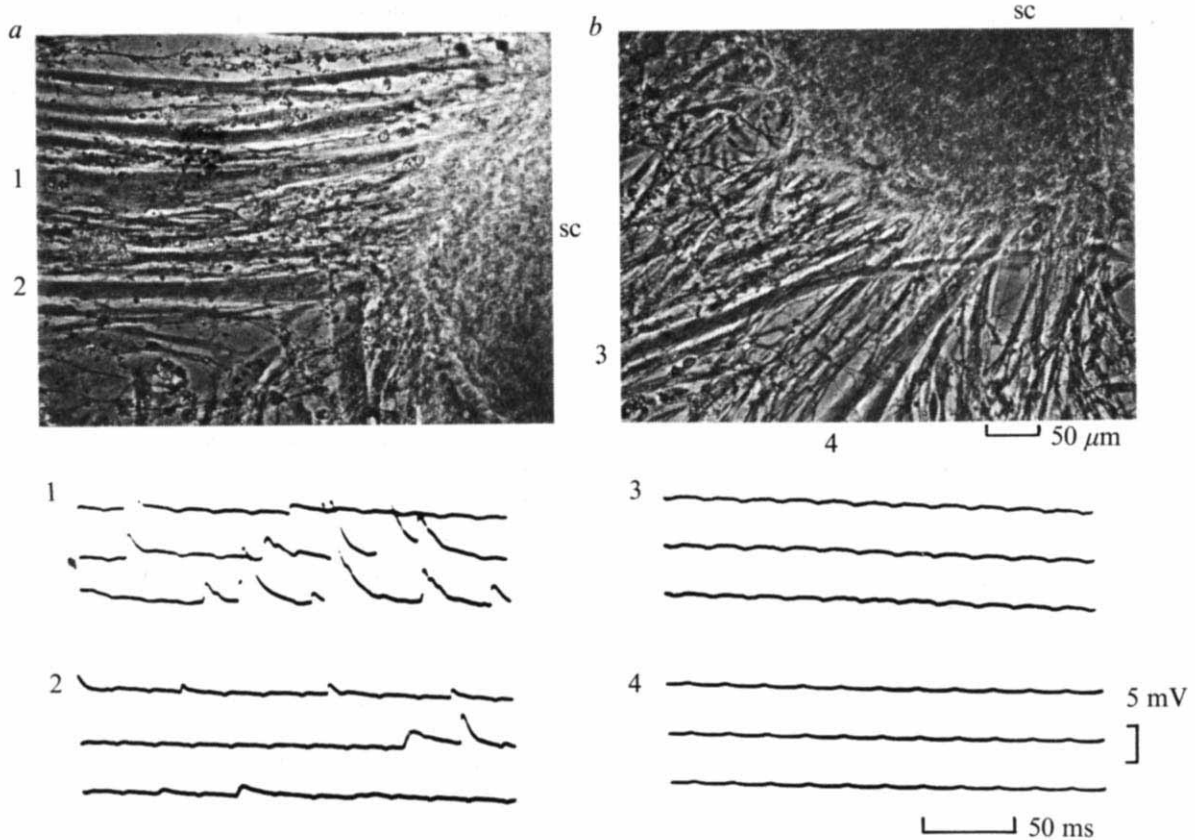


Fig. 1 Photomicrographs of cultures of skeletal muscle and the spinal cord (sc) from the chick embryo, incubated for 2 d. *a*, Without addition of glycolipid, and *b*, in the presence of 0.25 mM globoside. Potential records 1–4 were obtained from myotubes 1–4 respectively, as indicated in the photomicrographs. Spontaneous e.p.ps were obtained from myotubes 1 and 2 but not from 3 and 4.

muscular junction. The myotubes which were partially covered with the spinal cord explants or with numerous nerve processes were chosen for electrode penetration. Myotubes with a recorded resting potential lower than -40 mV were not included in the study. Four independent myotubes were sampled for each explant and 10–12 myotubes were studied in each culture dish. Percentages of myotubes generating spontaneous e.p.ps were compared for seven glycolipid samples (Table 1) at a concentration of 0.5 mM, except for sulphatide. Sulphatide at concentrations over 0.1 mM was toxic to growth of muscle cells and nerve processes, so the effect on junction formation was studied at 0.05 mM. The results are summarised in Table 1. Compared with the control cultures grown without glycolipids, galactosylceramide and sulphatide showed high values and glucosylceramide was without change. Cultures grown with haematoside, the mixture of gangliosides, G_{M1} and globoside were lower in percentages of myotubes with e.p.ps than controls. This difference was statistically significant only for globoside and G_{M1} , although growth of nerve and muscle did not seem to be impaired (Fig. 1 and see later). Further analyses were mainly made using globoside.

Effects of globoside varied with concentration (Fig. 2); an inhibitory effect was observed at higher concentrations (0.25–0.5 mM), but junction formation was augmented at lower concentrations (8–63 μ M). Biphasic effects were also observed with the sugar chain of globoside, although the transition from facilitation to depression occurred at a higher concentration than with globoside (Fig. 2). The experiments to determine whether there was a direct effect of globoside on the growth of nerve and muscle are summarised in Table 2. Outgrowth of nerve processes was not affected. High concentrations (0.25–0.5 mM) of globoside sometimes impaired the growth of myotubes but resting potential, input resistance and acetylcholine sensitivity were the same in cells cultured with and without globoside. Globoside thus has a rather selective

effect on the junction formation. The incidence of junction formation was low in the presence of 0.5 mM G_{M1} , but high at 0.25 mM (myotubes with e.p.ps constituting 72% of 40 cells tested).

Globoside was also added to cultures for various times as shown in Table 3, at a constant concentration of 0.25 mM. In contrast with depression induced by exposure to globoside throughout the culture period (48 h), exposure to globoside

Fig. 2 Effects of globoside (○) and its sugar chain (●) on the development of neuromuscular junction in culture. Myotubes were cultured with the spinal cord for 2 d in the presence of various concentrations of test substances. Ordinate shows percentage of myotubes which were spontaneously generating e.p.ps. A dotted line indicates level of the control (55%, Table 1). The numbers of myotubes tested are shown in parentheses.

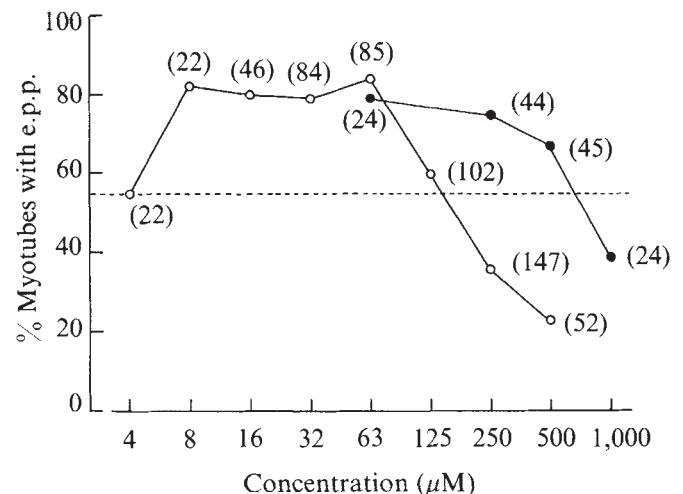


Table 2 Globoside effect on nerve and muscle cell culture growth

Globoside concentration (mM)	0	0.05	0.25
Process outgrowth from the spinal cord explants			
Density	1.7±0.11	1.4±0.17	1.6±0.13
Length	2.1±0.12 (46)	2.0±0.23 (24)	1.9±0.17 (28)
Electrical properties of myotubes			
Resting potential (mV)	-56.5±18.1 (184)	-57.9±19.6 (71)	-54.7±20.9 (155)
Input resistance (mΩ)	2.5±1.0 (3)		2.6±0.7 (3)
Acetylcholine sensitivity (mV nC ⁻¹)	425±436 (6)		483±287 (13)

Explants of the spinal cord from 6 d chick embryo were cultured for 2 d without muscle cells, with or without globoside. The extent of process outgrowth in fibre density and length was rated on an arbitrary scale of 0–3, a score of 3 indicating the maximum (modified from Mizel and Bamberg¹⁷). Electrical properties of myotubes were obtained from the same cultures as for the junction formation. Input resistance was measured by two intracellular electrode method. Acetylcholine was applied electrophoretically through micropipettes. In parentheses are numbers of the spinal cord explants or myotubes tested. No statistical significance was found when compared with the control (globoside, 0 mM).

during the first 12–24 h markedly potentiated junction formation. In these experiments the culture was repeatedly washed after the exposure, because even at a very low concentration globoside would affect the succeeding culture (see Fig. 2). Exposure for the first 6 h in a small number of experiments also showed mild augmentation of junction formation (not shown). Table 3 also shows that junction formation was low when globoside was applied later (12–24 h after plating the spinal cord). In a further experiment (not shown) globoside was applied to muscle culture for 24 h before the spinal cord was added. When studied after 2 d of coculture with the cord, e.p.ps were observed in 40% of 50 myotubes. Growth of myotubes was slightly impaired in these cultures. This suggests that the facilitatory effect was exerted on the nerve rather than on the muscle cells.

Table 3 Effect of time of globoside application (0.25 mM) on development of the e.p.ps in 48-h culture of muscle and the spinal cord

Exposure time (h)	No. of myotubes tested	Myotubes with e.p.ps (%)
0–48	147	36
0–12	86	85*
0–24	95	78*
12–48	72	40†
24–48	89	44†

**P* < 0.01 when compared with 0–48-h value by χ^2 test on 2×2 contingency tables.

†*P* > 0.05.

Judging from the onset of appearance of the spontaneous e.p.ps, neuromuscular junctions in these cultures were formed 12–48 h after plating the spinal cord (most being formed at 20–30 h). The fact that junction formation was stimulated by globoside during first 12 h of exposure suggests that, before junction formation, globoside or its sugar chain induces changes in nerve and muscle which resulted in subsequent facilitation of junction formation. Globoside is probably incorporated into cell membrane in a way analogous to that of ganglioside^{5,7}, although incorporation of globoside has not been studied. The globoside sugar chain, however, is probably not incorporated as a constituent of membrane globoside (as it is a saccharide, and not a glycolipid). Hence the facilitatory effect of globoside and its sugar chain may not necessarily be accounted for by an increased level of membrane globoside.

High concentrations of globoside were inhibitory when added at the time of the junction formation (12–48 h after plating of the spinal cord). It is possible that the inhibitory effect may overcome the facilitatory one which has been exerted in advance.

Development of the chick embryo neuromuscular junction *in vitro* was thus markedly affected by globoside and G_{M1}. Further investigation is required to elucidate the mechanism

of their action and also to find their role in the development of the neuromuscular system *in vivo*. The glycolipid composition of skeletal muscle is quite different from that of the nervous tissue¹⁶. The effects of other glycolipids on junction formation will be studied later.

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Antipsychotics block muscarinic acetylcholine receptor-mediated cyclic GMP formation in cultured mouse neuroblastoma cells

ANTIPSYCHOTIC drugs (for example, phenothiazines, butyrophenones) have side effects similar to those of atropine¹, suggesting that they block the muscarinic acetylcholine receptor. Receptor-binding studies with radioactively-labelled ligands have been used to study the muscarinic acetylcholine receptor and to show that antipsychotic drugs vary in their potency at displacing the ligand from binding sites in rat brain homogenates^{2,3}. In all receptor binding studies it is essential to demonstrate that binding data in fact reflect a drug-receptor interaction in a pharmacological sense⁴. These correlations are ideally determined using the same tissue for binding and for biological studies such as was done for certain polypeptide hormone receptors⁴ and for the β -

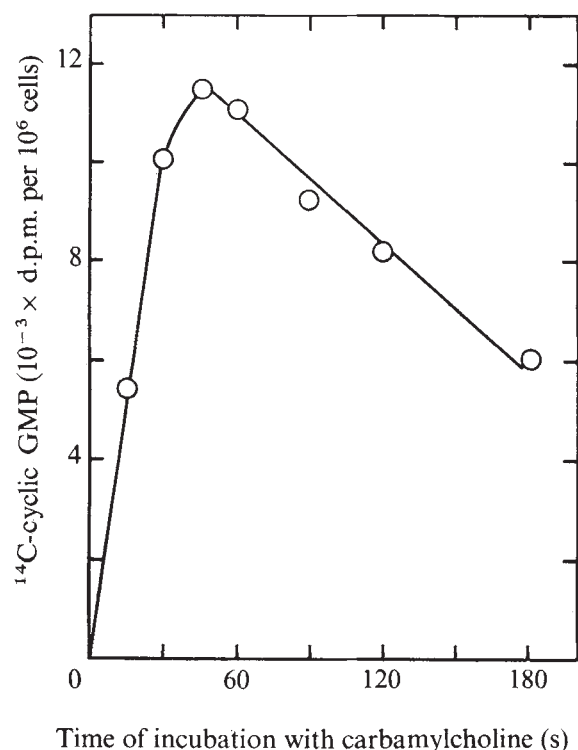


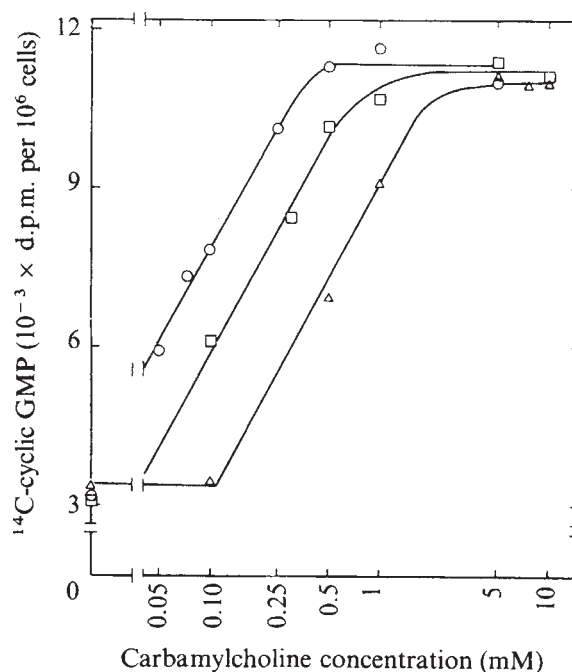
Fig. 1 The effect of carbamylcholine on cyclic GMP formation in cultured mouse neuroblastoma cells. Clone N1E-115 cells were cultured in Dulbecco-Vogt modification of Eagle's medium (high glucose, no pyruvate; Grand Island Biological) supplemented with 10% (v/v) foetal calf serum without antibiotics at 37°C in an atmosphere of 10% CO₂ and 90% humidified air. Cells were grown to confluency in flasks (75 cm² per 250 ml) with 20 ml of medium and maintained in stationary phase (requiring daily medium change) for 6 d before assay. For the assay cells were dissociated from the surface of flasks by incubation in a modified Puck's D₁ solution⁸, collected by low-speed centrifugation (500 r.p.m. in Damon/IEC CRU-5000 centrifuge) and resuspended at a density of 8×10^6 cells per ml in 2.0 ml of phosphate buffered saline (PBS)-HEPES solution containing 110 mM NaCl, 5.3 mM KCl, 1.8 mM CaCl₂, 1.0 mM MgCl₂, 2.0 mM Na₂HPO₄, 25 mM glucose, 50 mM sucrose and 20 mM HEPES *N*-2-hydroxyethyl-piperazine-*N*₂-ethane titrated to pH 7.4 with 1 N NaOH. The osmolality of this solution was 335–340 mOsm. The cell suspension was then placed in a 25-ml Erlenmeyer flask to which was added U-¹⁴C-GTP (specific activity 523 mCi mmol⁻¹) to give a final concentration of 5 μM (1.25 μCi ml⁻¹), and rotated at 37°C for 45 min at 80 r.p.m. Cells were then collected by low-speed centrifugation; washed three times with 10 ml of PBS-HEPES to remove extracellular radioactivity; and resuspended in 5 ml of PBS-HEPES containing 0.5 mM IBMX (Aldrich). The radioactive-cell suspension was then distributed in 250-μl aliquots into wells of a multi-well tray and incubated at 37°C for 30 min at 80 oscillations per min in a shaker bath. A solution of carbamylcholine (Sigma) was then added in 50 μl to give a final concentration of 1 mM. At the indicated times the reaction was stopped by the addition of 100 μl of ice-cold 50% (w/v) trichloroacetic acid (TCA). The TCA solution in each well was then sonicated briefly (3 s at setting no. 3. Ultrasonic Cell Disrupter, Kontes Glass) before the addition 0.02 μCi of 8-³H-cyclic GMP as internal standard. Each sample was then placed over an 0.8 × 8 cm column of Dowex 50 (AG 50 W-X2, 200–400 mesh, Bio-Rad) which had been washed with water. Each well was rinsed once with 500 μl of 5% TCA and this wash was placed over the column. Radioactive cyclic GMP was isolated from each column as described by Fujimoto *et al.*⁹ Recovery of ³H-cyclic GMP internal standard averaged 86%. The ¹⁴C-radioactivity in the fraction of the column containing ³H-cyclic GMP was identified as ¹⁴C-cyclic GMP by high voltage electrophoresis on Whatman No. 1 paper (25 mM NH₄HCO₃ buffer; 1 kV; 5 mA for 60 min) and thin-layer chromatography (PEI-cellulose in two solvent systems *n*-propanol 1 N LiCl (1/1) and 2 N formic acid/2 M LiCl (1/1). Of the recovered ¹⁴C-radioactivity, 86–94% electrophoresed or chromatographed with authentic cyclic GMP (Sigma). All time points were determined in duplicate. Corrections were made for recovery of the internal standard and the zero-time point value (5,800 d.p.m. per 10⁶ cells) was subtracted from each time point.

adrenergic receptor⁵. For the muscarinic acetylcholine receptor of nervous tissue, however, a direct correlation of binding data with pharmacological data has not been shown⁶. Thus, there remains a question whether the displacement by antipsychotic drugs of a radioactively-labelled muscarinic receptor antagonist from brain homogenates reflects blockade by these drugs of this receptor. We report here that antipsychotic drugs block the muscarinic acetylcholine receptor in cultured nerve cells.

For these studies we developed a technique to assay rapidly muscarinic acetylcholine receptor-mediated formation of cyclic GMP in intact cells of adrenergic mouse neuroblastoma clone N1E-115. We have studied this clone in cell culture as a model for the adrenergic neurone using biochemical, pharmacological and electrophysiological techniques⁷. The assay for cyclic GMP (to be described in detail elsewhere) involves radioactively-labelling intracellular pools of GTP and isolation of radioactively-labelled cyclic GMP over a cation exchange resin column.

The formation of ¹⁴C-cyclic GMP from ¹⁴C-GTP in living mouse neuroblastoma cells occurred rapidly after addition of carbamylcholine to the cells (Fig. 1). The peak of ¹⁴C-cyclic GMP was at about 45 s in the presence of the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX) (Fig. 1) and at about 30 s in the absence of IBMX (data not shown). Since stimulation of the formation of radioactively-labelled cyclic GMP in intact clone N1E-115 cells was inhibited by atropine ($K_B = 2 \times 10^{-9}$ M, E.R., unpublished data) and unaffected by hexamethonium (0.1 mM, E.R., unpublished data) we concluded that this effect was mediated through the muscarinic acetylcholine receptor. Others have found similar results with this clone¹⁰, autonomic ganglia and brain tissue¹¹. A possible role of muscarinic cholinergic regulation of cyclic GMP in synaptic transmission has been proposed¹¹.

Fig. 2 Effect of an antipsychotic drug on the carbamylcholine dose-response curve. Mouse neuroblastoma clone N1E-115 (subculture 14) was assayed for carbamylcholine-stimulated cyclic GMP formation as described in the legend to Fig. 1 except for the following modifications. Cells in PBS-HEPES with 0.5 mM IBMX were distributed in 250 μl aliquots to each well; 30 μl of PBS-HEPES was added with or without thioridazine (Sandoz) and the cells were incubated for 30 min before addition of carbamylcholine in 20 μl. There were approximately 3.1×10^5 cells and 224 μg of protein per well. Cell counts were determined with Coulter counter; protein was determined by the modification of the method of Lowrey *et al.*¹³, using bovine serum albumin as standard. ○, No antagonist; □, thioridazine 1×10^{-7} M; Δ, 5×10^{-7} M thioridazine.



Dose-response curves for carbamylcholine (Fig. 2) were shifted to the right in the presence of antipsychotic drugs (as for example, by the piperidine phenothiazine, thioridazine, Fig. 2). The parallel shift of the dose-response curve indicates competitive inhibition and allows the determination of the equilibrium dissociation constant for the antagonist-receptor complex from the equation $K_B = (DR - 1)/[B]$ where DR = dose ratio and $[B]$ = concentration of antagonist^{13,14}. In this manner, K_B values for a number of antipsychotic drugs were determined (Table 1). The potency of these antipsychotic drugs varied over a broad range. Thus, the most potent drug, clozapine (a dibenzodiazepine) was about 10^4 times more active than the least potent drug prochlorperazine (a phenothiazine). The rank order of potency of these drugs in our system was much the same as that obtained using receptor-binding studies with rat brain homogenates^{2,3}. Thus, using a biological assay for the muscarinic acetylcholine receptor, we have demonstrated that antipsychotic drugs competitively block this receptor, thereby blocking muscarinic receptor-mediated formation of cyclic GMP.

Table 1 Equilibrium dissociation constants (K_B) for antipsychotic drugs and the muscarinic acetylcholine receptor

Compound	(K_B) (M)
Clozapine	3×10^{-9}
Thioridazine	6×10^{-8}
Mesoridazine	6×10^{-8}
Promazine	2×10^{-6}
Chlorpromazine	2×10^{-6}
Fluphenazine	2×10^{-6}
Perphenazine	4×10^{-6}
Acetophenazine	4×10^{-6}
Haloperidol	7×10^{-6}
Trifluoperazine	2×10^{-5}
Prochlorperazine	4×10^{-5}

Each compound was tested at 2 different concentrations in a manner similar to that for Fig. 2. K_B values were calculated from the shift in the dose-response curves as described in the text. Average values are reported.

It is likely that *in vivo* antipsychotic drugs block both acetylcholine-stimulated guanylate cyclase activity and dopamine-stimulated adenylate cyclase activity. Effects of antipsychotic drugs on receptor-mediated formation of both cyclic GMP and cyclic AMP may relate to the therapeutic effects as well as the side effects (such as drug-induced Parkinsonism and tardive dyskinesia) of these compounds.

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Response of acetylcholine receptors to rapid photochemically produced increases in agonist concentration

SYNAPTIC transmission to muscle fibres and electroplaques takes place on a millisecond timescale. To study the molecular events leading to activation of the cholinergic receptor channel, we have developed a method to produce similarly rapid, spatially uniform steps of agonist concentration near intact postsynaptic membranes. *Electrophorus* electroplaques in a voltage-clamp apparatus were exposed to a solution containing a photochromic compound. Initially the compound was in a predominantly *cis* form, which had little effect on the membrane. During a brief light flash, some of the compound was isomerised to the *trans* isomer, which is a cholinergic agonist. As a result the membrane conductance increased along a timecourse which reflects the rate processes underlying activation of the cholinergic receptor channel.

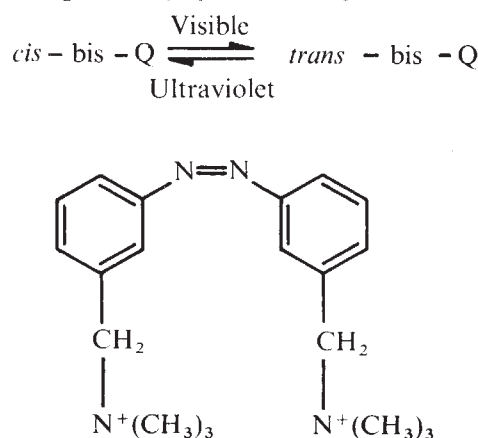
Bartels *et al.*¹ first described the photochemistry and pharmacology of 3,3'-bis-[α -(trimethylammonium)methyl]azobenzene (*bis-Q*; see Fig. 1). In confirmation of their results, we have found that a curare-sensitive conductance is induced in the innervated membrane by bath application of solutions containing *trans-bis-Q* (ref. 2). We have also exploited the fact that for a given concentration of any agonist the steady-state number of open receptor channels increases as the voltage is made more negative. After a voltage jump the agonist-induced current relaxes exponentially to a new equilibrium value; at low agonist concentrations the relaxation time constant equals the lifetime of the elementary change ('channel') caused by agonist^{3,4}. With *trans-bis-Q*, this time constant was about 11 ms at 10 °C and -150 mV, and about 2.5 ms at 24 °C and the same voltage. Roughly equal values are obtained with acetylcholine (ACh) itself in similar conditions.

Many quaternary ammonium compounds possessing aromatic groups produce an apparent non-competitive, voltage-dependent blockage of ACh receptors^{2,7}, but such effects are small or absent with *bis-Q* at the concentrations used here.

We have also confirmed the observation¹ that the *cis* isomer has much less effect on ACh receptors, although its precise pharmacology is uncertain because it has not yet been isolated in pure form. The *cis* and *trans* isomers are stable in the dark but readily interconvert when exposed to light of appropriate wavelengths (Fig. 1). This property affords the opportunity to observe the response to rapid 'agonist concentration jumps'.

Before such experiments the *bis-Q* solutions were converted to a predominantly *cis* form by exposure to 360-nm light until no further changes in optical density were noted at 318 nm. Bartels *et al.*¹ estimated that the resulting 'photostationary' state consists of 85% *cis* and 15% *trans* (active) isomer. Consequently, small agonist-induced currents appeared when this solution was allowed to equilibrate with the innervated face of an electroplaque; at

Fig. 1 *Bis-Q*, a photochromic agonist.



–150 mV these initial agonist-induced currents were roughly 2 mA cm^{-2} in the cell of Fig. 2a, and 5 mA cm^{-2} in Fig. 2b. After a voltage jump, relaxations of the agonist-induced current resembled those seen with low concentrations of *trans*-bis-Q. There are at least two components. A fast ($< 500 \mu\text{s}$) phase is kinetically not resolvable and accounts for 10–25% of the relaxation amplitude. This component probably results from instantaneous rectification in the small number of channels opened by *trans*-bis-Q at positive potentials⁴. The slower part of the relaxation follows an exponential timecourse, and arises because the number of open receptor channels increases after the voltage jump⁴.

We then compared the behaviour of the conductance during voltage-jump relaxations and agonist concentration-jump relaxations induced by light flashes (Fig. 2). An energy of 75 J was discharged through a xenon-filled 'short-arc' flash tube. The light was led through a fibre optic bundle to the pool, roughly a 1-cm cube, bathing the innervated face of the voltage-clamped electroplaque. The fibre bundle filtered out most of the ultraviolet light. Between light flashes the preparation was kept in the dark. When the pool contained *cis*-bis-Q, we found with spectral measurements that each flash converted about 2.5% of the *cis* to the *trans* isomer.

Although 'voltage-jump' relaxations occurred with all known agonists^{3–6}, conductance changes following a light flash were observed only when the solution contained *cis*-bis-Q and not in the presence of agonists, such as suberyldicholine or pure *trans*-bis-Q, which are photostable at the wavelength used. The agonist concentration jump produces an increase of membrane conductance. (We have not yet succeeded in generating ultraviolet flashes strong enough to produce rapid conductance decreases due to *trans*→*cis* isomerisation.) Like the voltage-jump relaxations, the concentration-jump relaxation amplitudes are reversibly diminished about fivefold by (+)-tubocurarine ($1 \mu\text{M}$). Thus there seems little doubt that the agonist concentration-jump relaxations reflect a rate-limiting process in the activation of channels controlled by nicotinic acetylcholine receptors.

As shown in Fig. 2, the conductance increase is substantially complete within the first few milliseconds following the flash and

stable at least until the next episode (4 s later in the experiment of Fig. 2, several minutes later in other experiments). The voltage- and concentration-jump relaxations have similar timecourses at a given membrane potential and temperature. Presumably the rate of *cis*→*trans* isomerisation is at least as fast as the flash, which lasted about 2 ms; after the flash the agonist concentration-jump relaxations followed an exponential timecourse. For the six traces of Fig. 2a, measured at 10°C , the major, exponential component of the voltage-jump relaxation had a time constant of $6.94 \pm 0.29 \text{ ms}$ (mean $\pm$ s.e.). The agonist concentration-jump relaxation had a time constant of $6.80 \pm 0.28 \text{ ms}$. At 24°C (Fig. 2b), the values were $1.78 \pm 0.09 \text{ ms}$ for the voltage jump and $1.75 \pm 0.14 \text{ ms}$ for the agonist concentration jump. On average, the time constants decreased by about 1% after each flash for the first 20 flashes; this observation is not surprising since voltage-jump relaxation times decrease with increasing agonist concentration^{3,4}. The time constants vary markedly with temperature, suggesting that channel opening and closing are rate-limited by a chemical, probably macromolecular, process rather than by simple diffusion of newly created agonist molecules toward their site of action.

Several electrophysiological techniques are now available for probing the opening and closing kinetics of acetylcholine receptor channels. Thus it is possible to compare (a) the decay of neurally evoked postsynaptic currents and of spontaneous miniature postsynaptic currents^{3,4,8–10} (b) the duration of single-channel currents¹¹; (c) the power spectrum of ACh-induced conductance fluctuations^{5,12,13}; (d) voltage-jump relaxations^{3–6}; and (e) the present agonist concentration-jump relaxations. For the first two experimental procedures, kinetic data are dominated by the closing rates; data from the last three procedures sometimes have comparable contributions from the opening rates as well⁴. The time constants derived from all these experiments agree well in comparable conditions of agonist, concentration, voltage, and temperature. These facts suggest that the opening and closing of the channel are governed either by true first-order processes such as intramolecular isomerisations, or by pseudo-first-order processes such as binding of agonist molecules whose concentration remains essentially constant during the measurement. To distinguish among these possibilities, we require improvements in the agonist concentration-jump technique coupled with similarly rapid measurements of binding and of conformational changes¹⁴.

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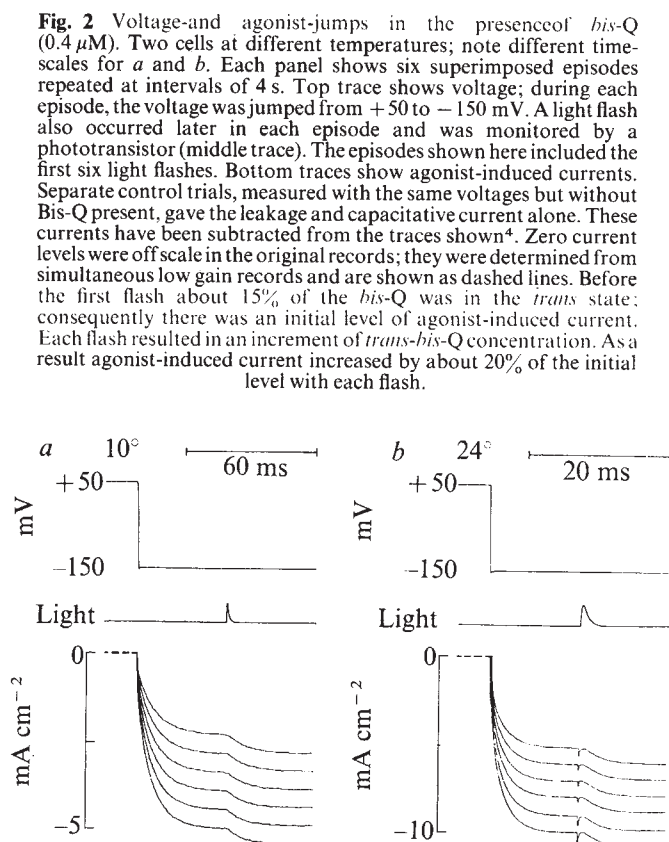


Fig. 2 Voltage- and agonist-jumps in the presence of *bis*-Q ($0.4 \mu\text{M}$). Two cells at different temperatures; note different time-scales for a and b. Each panel shows six superimposed episodes repeated at intervals of 4 s. Top trace shows voltage; during each episode, the voltage was jumped from +50 to –150 mV. A light flash also occurred later in each episode and was monitored by a phototransistor (middle trace). The episodes shown here included the first six light flashes. Bottom traces show agonist-induced currents. Separate control trials, measured with the same voltages but without *Bis*-Q present, gave the leakage and capacitive current alone. These currents have been subtracted from the traces shown⁴. Zero current levels were off scale in the original records; they were determined from simultaneous low gain records and are shown as dashed lines. Before the first flash about 15% of the *bis*-Q was in the *trans* state; consequently there was an initial level of agonist-induced current. Each flash resulted in an increment of *trans*-*bis*-Q concentration. As a result agonist-induced current increased by about 20% of the initial level with each flash.

Release of dopamine *in vivo* from cat substantia nigra

THE central aminergic neurones present some original characteristics. By means of quantitative autoradiographic studies, it has been demonstrated that serotonergic terminals projecting into the cerebral cortex and the locus coeruleus exhibit very few classical synaptic contacts^{1,2}. This has also been seen with cortical noradrenergic terminals¹. It has been suggested that the amines could be released not only in synapses, but also from varicosities devoid of synaptic junctions: they could thus act as neuromodulators at some distance from the release sites. It has also been proposed that dopamine could be released from dendrites of nigrostriatal dopaminergic neurones³. Indeed, a release of endogenous dopamine (Cuello, personal communication) or of labelled dopamine^{4,5} previously taken up in tissues, has been demonstrated using slices of the rat substantia nigra exposed to a high concentration of potassium (30 mM). This process was calcium dependent. An indication of possible dendritic release was also obtained indirectly *in vivo* by measuring dopamine metabolites in the rat substantia nigra⁶. Dihydroxyphenylacetic acid and homovanillic acid levels were increased after stimulation of the medial forebrain bundle, which contains axons of various ascending aminergic pathways including the nigrostriatal dopaminergic system. Therefore, dopamine could be released not only from nerve terminals in the striatum, but also from dendrites in the substantia nigra. If the latter mechanism was occurring in physiological states, it could have important implications for the understanding of the regulatory processes involved in the control of the activity of the dopaminergic neurones. It thus seemed necessary to demonstrate directly an *in vivo* release of dopamine in the substantia nigra.

Experiments were carried out in halothane-anaesthetised cats. A push-pull cannula was inserted into the substantia nigra (stereotaxic coordinates: $A = 3.5$; $L = 4$; $H = -4$) allowing the superfusion of the structure with artificial cerebro-spinal fluid containing 20–25 μCi per 500 μl L-3, 5-³H-tyrosine (40–50 Ci mmol^{-1} , Radiochemical Centre, Amersham, UK) at a rate of 500 μl per 15 min. ³H-dopamine formed and released extraneuronally, was estimated in serial 15-min superfusate fractions using an isolation procedure as described previously⁷. In some experiments the push-pull cannula was introduced into the left caudate nucleus ($A = 16$; $L = 5$; $H = +5$). This experimental approach for the study of dopamine release from nerve terminals has already been used successfully⁸.

When the tip of the push-pull cannula was positioned precisely in the substantia nigra, as revealed by postmortem histological control, ³H-dopamine could be easily estimated in the superfusates. One hour after the beginning of the experiments, ³H-dopamine release reached a steady-state level (about 20 times the blank value) which persisted during the entire course of the superfusion (Fig. 1). As checked by cochromatography, ³H-

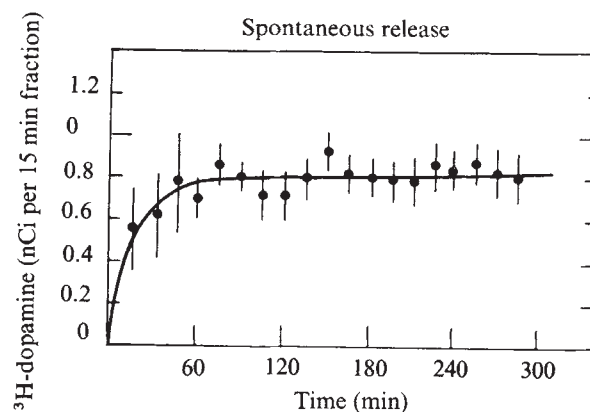


Fig. 1 Spontaneous release of ³H-dopamine from substantia nigra in halothane-anaesthetised cat. ³H-dopamine was estimated in serial superfusate fractions (15 min each) collected from a push-pull cannula during the continuous local superfusion of the left substantia nigra with an artificial CSF containing L-3, 5-³H-tyrosine (20–25 μCi per 500 μl for 15 min) in halothane-anaesthetised cats. Data represent mean $\pm$ s.e.m. of results obtained in four experiments.

dopamine was the only labelled catecholamine released (Fig. 2). The quantity of ³H-dopamine spontaneously released into the substantia nigra was 3–4 times lower than that found in comparable experiments carried out in the left caudate nucleus (Table 1).

We attempted to demonstrate that various treatments could influence the spontaneous release of endogenously synthesised ³H-dopamine in the substantia nigra. When potassium (30 mM) was introduced for 15 min on the CSF, the release of ³H-dopamine was markedly enhanced. This stimulating effect was of short duration: the quantities of ³H-dopamine released returned to the steady-state value as early as 15 min after the end of the potassium application (Fig. 3A).

(D)-amphetamine and benztropine markedly enhanced the release of ³H-dopamine synthesised from L-3, 5-³H-tyrosine in the caudate nucleus^{8–10} by stimulating the transmitter release and inhibiting its reuptake. It was therefore of interest to determine whether these drugs were exerting similar effects in the substantia nigra, rich in dopaminergic dendrites. Both drugs, introduced for 15 min into the superfusing fluid immediately enhanced the transmitter release. The effect was of short duration with (D)-amphetamine, but persisted with benztropine even though the drug had been removed from the artificial CSF (Fig. 3b and c).

These results provide the first direct demonstration of the spontaneous and evoked release of dopamine endogenously synthesised in the substantia nigra in physiological states. It can be assumed that the transmitter is released from dendrites, as suggested initially by Björklund and Lindvall³ on the basis of

Table 1 Effects of TTX on ³H-dopamine release from caudate nucleus and substantia nigra of the cat

Structure		n	nCi per 15 min successive fractions			
			0–15 min	15–30 min	30–45 min	45–60 min
Caudate nucleus	Control	4	3.14 $\pm$ 0.40	3.27 $\pm$ 0.28	3.24 $\pm$ 0.24	3.21 $\pm$ 0.36
	TTX (5×10^{-7} M)	4	2.23 $\pm$ 0.17	1.92 $\pm$ 0.13*	1.62 $\pm$ 0.26*	1.49 $\pm$ 0.10*
	% Change		–29%	–41%	–50%	–54%
Substantia nigra	Control	3	0.84 $\pm$ 0.10	0.85 $\pm$ 0.11	0.80 $\pm$ 0.09	0.82 $\pm$ 0.12
	TTX (5×10^{-7} M)	3	1.42 $\pm$ 0.16*	1.06 $\pm$ 0.14	1.14 $\pm$ 0.10*	1.03 $\pm$ 0.13
	% Change		+69%	+25%	+43%	+26%

A discrete area of either the caudate nucleus or the substantia nigra of halothane-anaesthetised cats was superfused continuously with an artificial CSF containing L-3, 5-³H-tyrosine (20–25 μCi per 500 μl for 15 min) using a push-pull cannula. ³H-dopamine was estimated in 15 min successive superfusate fractions. TTX (5×10^{-7} M) was added for 1 h to the superfusion fluid 180 min after the onset of the superfusion. Data represents the mean $\pm$ s.e.m. of ³H-dopamine quantities found in the four fractions during TTX application and in corresponding fractions of control animals.

* $p < 0.05$ compared with respective control.

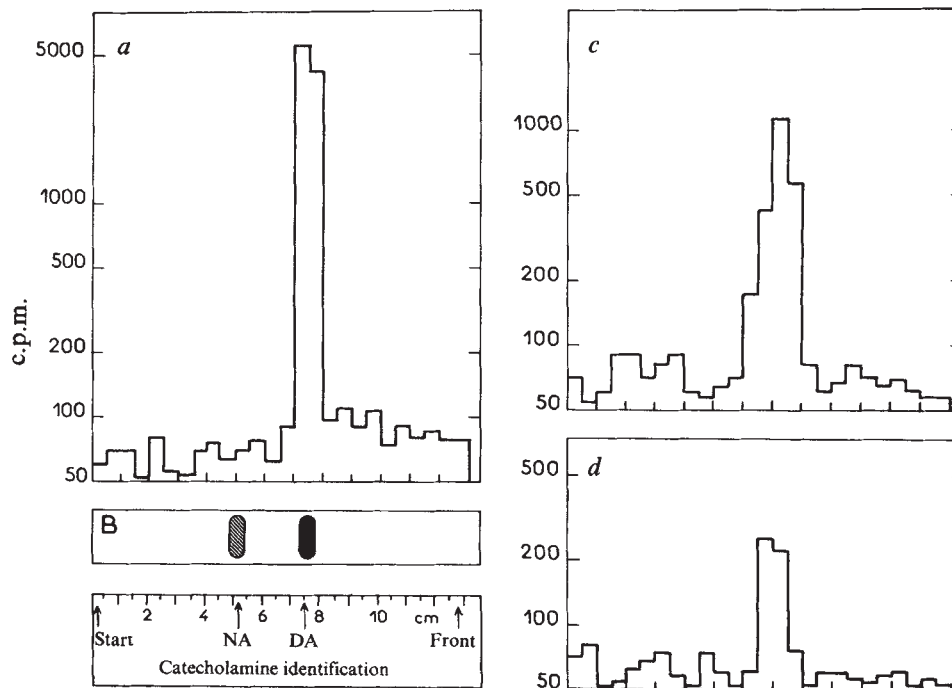


Fig. 2 Identification of the ^3H -catecholamine released from the substantia nigra. ^3H -catecholamines in superfusates were isolated from ^3H -tyrosine and ^3H -metabolites by Amberlite ion-exchange chromatography and adsorption on alumina columns. Hydrochloric acid eluates of alumina columns corresponding to successive samples were pooled and submitted to thin-layer cochromatography on cellulose plates using *n*-butanol, acetic acid and water (4:1:2 v/v) as solvent. *a*, ^3H -dopamine standard; *b*, spots of the carriers as revealed by ninhydrin; *c*, ^3H -dopamine identification in pooled samples collected during the superfusion of the caudate nucleus; *d*, ^3H -dopamine identification in pooled samples collected during the superfusion of the substantia nigra. DA, Dopamine; NA, noradrenaline.

histochemical studies. Indeed, these authors observed a dopamine-containing varicose dendritic network well organised in the pars reticulata of the rat substantia nigra. Our results confirm and extend the *in vitro* findings of Geffen *et al.*⁵ and Cuello and Iversen⁴. ^3H -dopamine release was not only enhanced by potassium, but also by drugs affecting the release and/or re-uptake of dopamine in nerve terminals. It seems that dopamine released from dopaminergic dendrites can be partly inactivated by a potent

re-uptake process, as observed in nerve terminals. The presence of an active dopamine uptake process in dendrites has already been suggested in biochemical⁵, histochemical³ and autoradiographic studies^{11,12}. Exogenous dopamine can be taken up in slices of the rat substantia nigra⁴ and visualised in dendrites³; labelled dopaminergic dendrites were seen after an intraventricular^{11,12} or local injection of ^3H -catecholamines⁴. The dopaminergic dendrites contain tyrosine hydroxylase as indicated by a recent

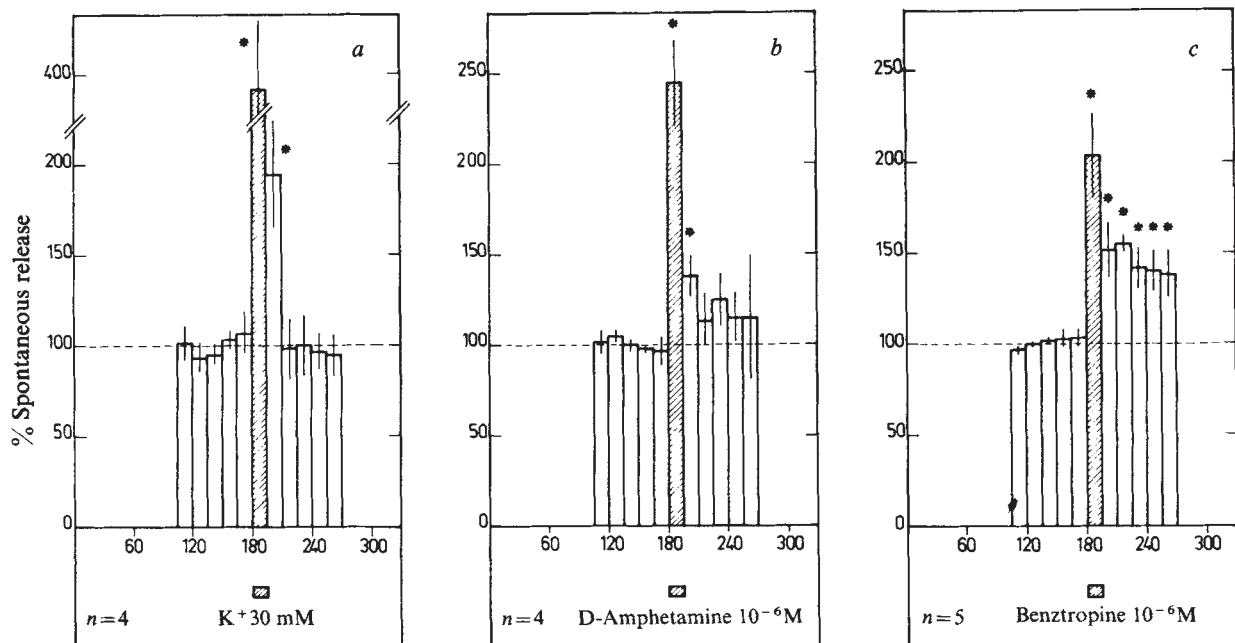


Fig. 3 Effects of K, (D)-amphetamine and benztropine on release of ^3H -dopamine from cat substantia nigra. A discrete area of the left substantia nigra was continuously superfused with artificial CSF containing L-3,5- ^3H -tyrosine (20–25 μCi per 500 μl for 15 min) using a push-pull cannula. The collection of superfusates (15 min each) for ^3H -dopamine estimation started 105 min after the beginning of the superfusion. Three hours after the onset of the superfusion, K (30 mM), (D)-amphetamine (10^{-6} M) or benztropine (10^{-6} M) was added for 15 min (hatched bars) to the medium introduced into the substantia nigra. In each animal, ^3H -dopamine in each successive fraction was expressed as a percentage of an average spontaneous release calculated from the five fractions collected before the drug application (105–180 min). Data are mean $\pm$ s.e.m. of results obtained with groups of *n* animals. **P* < 0.05 when compared with corresponding control values obtained in four animals.

immunohistochemical study¹³. Our results suggest that the local rate of the transmitter synthesis is important and can efficiently supply the release process. In fact, the steady-state level of ³H-dopamine synthesised from L-3,5-³H-tyrosine was reached even faster than in the caudate nucleus⁸.

The push-pull cannulae used allowed the superfusion of an area of approximately 2.6 mm³. Therefore, ³H-dopamine released could originate from the pars reticulata as well as from the pars compacta. It cannot be excluded that ³H-dopamine is partly released from dopaminergic cell bodies or axonal collaterals, or even from hypothetical dopaminergic intrinsic neurones. In any case, the release of dopamine at the level of the substantia nigra seems to be somewhat different from that observed in nerve terminals. This could be shown using tetrodotoxin (TTX). By blocking sodium channels, TTX suppressed the nerve impulse flow and subsequently the release of transmitters from nerve terminals. Thus, when TTX was introduced into the CSF superfusing the caudate nucleus, the release of ³H-dopamine was reduced by about 50%, as shown previously⁸. Surprisingly, an opposite effect was observed when TTX was introduced into the substantia nigra. In most superfusate fractions, the release of ³H-dopamine was significantly increased (Table 1). This original finding may be interpreted as follows: TTX, by blocking nerve impulse flow in afferences, may suppress tonic inhibitory influences on the activity of dopaminergic neurones. It obviously cannot suppress, however, the release of dopamine from dendrites as seen in nerve terminals, since ³H-dopamine release was increased in the presence of TTX. Therefore, it may be suggested that dopamine release from dendrites is not impaired by blocking sodium channels.

Dopamine released into the substantia nigra seems to have a physiological role. On the basis of microiontophoretic electrophysiological studies, Aghajanian and Bunney¹⁴ postulated that the amine inhibits the activity of dopaminergic cells by acting on dopaminergic autoreceptors. This dopamine effect is supported by recent experiments using our cat preparation. Dopamine (10⁻⁷ M) introduced into the substantia nigra inhibited the release of newly synthesised ³H-dopamine in the ipsilateral caudate nucleus¹⁵. This effect was also observed with concentration of (D)-amphetamine and benztropine similar to those used in the present study. Since dendrodendritic contracts have been observed in the substantia nigra^{4,16}, it can also be speculated that dopamine released from dendrites may affect the activity of neighbouring dopaminergic neurones: lateral inhibition is a process which has been extensively described by neurophysiologists¹⁷. Finally, a dopamine-sensitive adenylate cyclase has been found in the substantia nigra^{18,20}, particularly in the pars reticulata²⁰. Lesion studies have indicated that this adenylate cyclase system is not located in dopaminergic neurones but on descending neuronal afferents²⁰ which are presumably GABA-ergic or substance P-ergic⁴. The dopamine postsynaptic receptors associated with this dopamine-sensitive adenylate cyclase could be influenced by dopamine released from dopaminergic dendrites. It can thus be postulated that local interneuronal feedback processes participate in the control of the dopaminergic neuronal activity. Dopamine released *in vitro* from dopaminergic dendrites may modulate nigral afferents at a presynaptic level.

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High affinity uptake of glutamate in terminals of corticostriatal axons

GLUTAMIC ACID (glu) may be the transmitter of a large proportion of excitatory synapses in the brain¹, but glu is also important in cell metabolism, and the apparent lack of biochemical 'markers' associated with the role of glu as a synaptic transmitter has until recently hampered the localisation of potential 'glutamergic' neurones. High affinity uptake of glu^{2,3} is, however, highly specific² and seems to be selectively localised in the excitatory granular cell terminals in the cerebellum⁴, and in three systems of excitatory nerve endings in the hippocampal formation, as shown by autoradiography⁵ and quantitative measurements⁶. In the conditions used in these studies, glial uptake of glu^{7,8} was found not to be quantitatively important. In the hippocampal systems, iontophoretic studies⁹ and release experiments¹⁰ strongly suggest that glu, and/or aspartic acid (asp), may be the transmitter. It thus seems that high affinity uptake of glu may be useful as a marker for putative glutamergic and/or aspartergic nerve endings. The question of whether the uptake of [³H]glu, as measured *in vitro*, represents net accumulation or homoeostasis¹¹ is not crucial in the present context. The neostriatum (nucleus caudatus-putamen) receives a large excitatory projection from the neocortex^{12,13}. Iontophoretic studies suggest that this could use glu or asp as its transmitter^{1,14}. We here demonstrate that high affinity uptake of glu is selectively reduced in the neostriatum after lesions in the neocortex.

Male Wistar rats (250 g) were used. The frontal cortex, between the frontal pole and the frontoparietal suture, was removed down to white matter by suction through a fine needle under microscopic guidance. In sham-operated animals the skull was opened, but no lesion made; unoperated controls were only anaesthetised. After 7 or 14 d the animals were decapitated and frontal slices (about 1 mm) of the brains were prepared in the cold on a Sorvall tissue sectioner. The caudatus-putamen (avoiding the nucleus accumbens) was dissected from the rostral 1–2 slices containing the nucleus to obtain samples receiving cortical afferents predominantly from the lesioned part of the cortex¹². Histological preparations from other operated animals showed that the caudatus-putamen was not directly damaged by the lesions. The dissected samples were weighed on a Mettler microbalance and homogenised in 50 volumes 0.32 M sucrose solution with 5 mM Na phosphate buffer, pH 7.4, in a glass-Teflon homogeniser, to produce suspensions containing nerve ending particles.

High affinity uptakes of glu and γ -aminobutyric acid

Table 1 Selective reduction in high affinity uptake of glu in neostriatum after cortical lesions

Sample	n	Uptake of glu	Uptake of GABA	glu decarboxylase	Choline acetyltransferase	DOPA decarboxylase
L	6	113 ± 12*	30 ± 3	1,970 ± 180	3,590 ± 210	863 ± 72
N	6	193 ± 14†	34 ± 3	1,740 ± 120	3,580 ± 310	870 ± 118
C	7	239 ± 10	32 ± 2	1,800 ± 160	3,480 ± 370	913 ± 53

Results are presented as nmol/min/g protein ± s.e.m. of *n* samples.

L and N, lesioned and contralateral side, respectively, in operated animals; C, right and left sides in sham-operated (2) and unoperated animals (2).

**P* < 0.001 compared with C; *P* < 0.005 compared with N (Student's *t* test, both tails).

†*P* < 0.025 compared with C. Other differences were non-significant (*P* > 0.2).

(GABA) were measured by incubating 5 µl homogenate with 500 µl Krebs-phosphate solution at 25 °C for 3 min⁶. The reaction was started by adding 50 µl Krebs solution containing L-[3-³H]glu (New England Nuclear, Boston, Massachusetts, 15.8 Ci mmol⁻¹, diluted with unlabelled monosodium glutamate to give a specific radioactivity of 1.14 Ci mmol⁻¹) and [U-¹⁴C]-γ-aminobutyric acid (Radiochemical Centre, Amersham, UK, 224 mCi mmol⁻¹) to give final concentrations of 1 µM. Separate studies have shown that GABA and glu do not interact with each other's uptake in such conditions^{2,15}. After incubation the samples were diluted with 4 ml ice-cold 0.9 NaCl, collected on Millipore filters (0.45 µm), and rinsed with another two portions of 4 ml NaCl. The filters were eluted in 1.2 ml 10% Triton X-100 and counted with 10 ml Diluene (Packard) in a Packard liquid scintillation spectrometer (model 3380) fitted with an absolute activity analyser (model 544) giving d.p.m. for ³H and ¹⁴C separately. Samples incubated at 0 °C, but otherwise treated identically, served as blanks. Experimental and control samples were run together. Uptake of glu and GABA were linear with the amount of striatal tissue up to at least 100 µg wet weight. Protein¹⁶, glutamate decarboxylase¹⁷, choline acetyltransferase¹⁸ and DOPA decarboxylase¹⁹, were determined essentially as described previously.

After lesions of the frontal cortex there was a selective reduction in glu uptake in the nucleus caudatus-putamen, by 41% when the operated side was compared to the contralateral side, or by 53% compared with unlesioned animals (Table 1). The slight reduction on the side contralateral to the lesions compared with unlesioned animals is probably due to the fact that the corticostriatal projection is partly crossed²⁰. The reduction in glu uptake on the lesioned side was somewhat larger at 14 d (64% reduction compared with intact controls, 2 animals) than at 7 d survival time (49% reduction, 4 animals), but the results were pooled in Table 1. The lack of change in GABA uptake suggests that the reduction is not due to an unspecific interference with uptake processes. The absence of change in markers for alleged GABAergic (glu decarboxylase, GABA uptake), cholinergic (choline acetyltransferase) and dopaminergic neurones (DOPA decarboxylase) demonstrates that the reduction in glu uptake is not due to a general effect on neurones in the caudatus-putamen. The possibility that the selective reduction in glu uptake could be due to trans-synaptic changes—for example, in other excitatory afferents—can not be completely ruled out, but seems rather unlikely.

Since the acidic amino acids, glu and asp, have similar or identical high affinity uptake systems^{3,4}, and since glu uptake, measured in our conditions, seems to be a useful marker for nerve endings utilising acidic amino acids as transmitters^{4,6}, our results suggest that the neocortico-neostriatal projection could use glu or asp as its transmitter.

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Benzo[a]pyrene 7,8-dihydrodiol is more carcinogenic than benzo[a]pyrene in newborn mice

THE pioneering research by James and Elizabeth Miller relating the carcinogenic effects of many chemicals to the metabolism of these compounds to reactive intermediates which bind to critical cellular components^{1,2} led to studies of the nature and chemical structure of these reactive metabolites (ultimate carcinogens) and their metabolic precursors (proximate carcinogens). The environmental carcinogen benzo[a]pyrene (BP) is metabolised by microsomal enzymes to arene oxides, phenols and dihydrodiols. Metabolic activation of the BP metabolite (±)-*trans*-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene (BP 7,8-dihydrodiol) by liver microsomes resulted in more binding to DNA than did the metabolic activation of BP (ref. 3). The reactive metabolite involved in the binding to DNA was a *trans*-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydro BP (BP 7,8-diol-9,10-epoxide) (ref. 4). Both stereoisomers of this diol epoxide, which are enzymatically formed from BP 7,8-dihydrodiol (refs 5–8), were highly mutagenic to bacterial and mammalian cells^{9–15} suggesting that a BP 7,8-diol-9,10-epoxide might be an ultimate carcinogenic metabolite of BP. We report here that administration of BP 7,8-dihydrodiol to newborn mice caused more malignant lymphomas and pulmonary adenomas than did administration of benzo[a]pyrene. This is the first demonstration of a metabolite of a polycyclic aromatic hydrocarbon that is more carcinogenic than the parent compound.

Table 1 Carcinogenic activities of BP, BP 7,8-dihydrodiol and diol epoxide-2 in newborn mice

Treatment	Total dose (nmol)	Sex	No. of survivors at 25 d	No. of animals autopsied	Age 4-17 weeks		No. of animals autopsied	Age 18-24 weeks		
					No. of animals with:			No. of animals with:		
					malignant lymphoma	lung adenomas		malignant lymphoma	lung adenomas	other tumours
Control	0	♂	34	—	—	—	32	0	2	
		♀	17	—	—	—	16	0	0	
		Total	51	—	—	—	48	0	2	
BP	1400	♂	26	—	—	—	24	0	18	*
		♀	24	1	0	1	20	0	19	
		Total	50	1	0	1	44	0	37	
BP 7,8-dihydrodiol	1400	♂	24	9	5	3	12	8	11	†
		♀	40	17	12	5	12	10	11	‡
		Total	64	26	17	8	24	18	22	
Diol epoxide-2	28	♂	17	—	—	—	17	0	11	
		♀	20	—	—	—	20	0	19	
		Total	37	—	—	—	37	0	30	

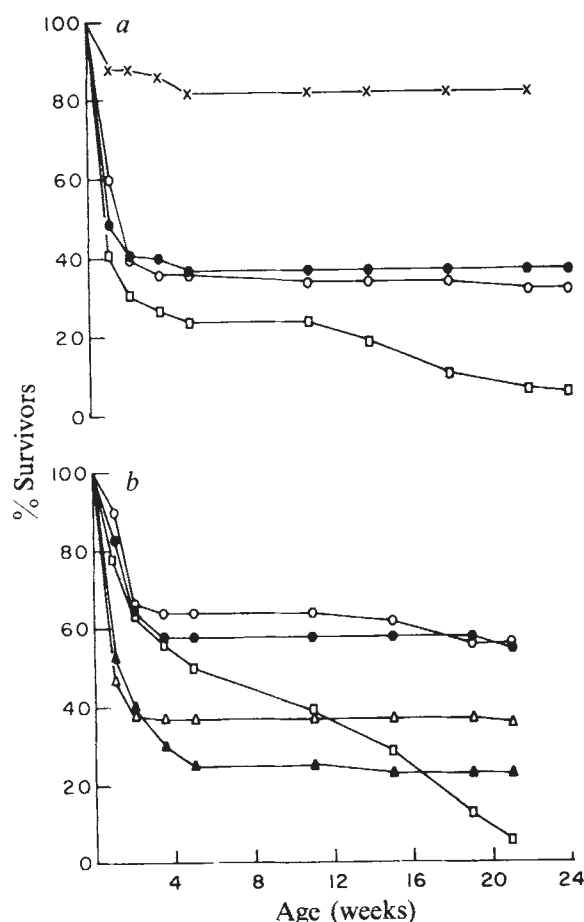
*Two mice with fibroma of the skin.

†Two mice with skin papillomas.

‡One mouse with fibroma of the skin.

Swiss Webster mice [BLU:Ha (ICR)] were injected i.p. with BP and BP derivatives. One-seventh of the indicated total dose of hydrocarbon was given on d 1 of life, two-sevenths on d 8 and four-sevenths on d 15. Control animals were given the vehicle, DMSO. The mice were weaned when 25 d old. The data were obtained from the survivors of experiments 1 and 2 described in Fig. 1. Animals more than 25 d old which died during the course of the study and animals killed at the end of the study (when the animals were 21-24 weeks old) were subjected to histological examination. A few animals which died after weaning were cannibalised and could not be examined.

Fig. 1 Toxicity of benzo[*a*]pyrene derivatives in newborn mice. Swiss-Webster mice [BLU:Ha (ICR)] were injected i.p. with BP or BP derivatives. One-seventh of the total dose of each compound was given on d 1 of life, two-sevenths on d 8 and four-sevenths on d 15. In experiment 1 (a), 50 animals were each injected with 1,400 nmol of BP (○), 70 animals with 1,400 nmol of BP 7,8-dihydrodiol (□), 70 animals with the vehicle (DMSO) (●) and 50 animals were untreated controls (×). In experiment 2 (b), 50 animals were treated with the above dose of BP, 80 animals received the above dose of BP 7,8-dihydrodiol and 40 animals were given DMSO. Diol epoxide-1 (28 nmol) (▲) was injected into 40 animals, and diol epoxide-2 (28 nmol) (△) was injected into 100 animals. The mice were weaned when 25 d old.



One of the stereoisomeric BP 7,8-diol-9,10-epoxides ((±)-*trans*-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydro BP; diol epoxide-2; also termed diol epoxide I (ref. 11) or anti-isomer (ref. 16)) produced about the same number of pulmonary adenomas as a 50-fold higher dose of BP.

BP 7,8-dihydrodiol, diol epoxide-1 [(±)-*trans*-7β,8α-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydro BP; also termed diol epoxide II (ref. 11) or syn isomer (ref. 16)] and diol epoxide-2 were synthesised as previously described^{17,18}, and all chemicals were of analytical-grade purity. Pregnant Swiss-Webster BLU:Ha (ICR) mice (Blue Spruce Farms) delivered their babies within 2-5 d after arrival in our laboratory. The healthiest-looking newborns were selected, and each mother was left with 10 babies. BP and BP 7,8-dihydrodiol were dissolved in anhydrous dimethylsulphoxide (DMSO) (ref. 10) at a final concentration of 200 nmol per 5 μl. The compounds were injected intraperitoneally (i.p.) as follows: 200 nmol on the first day of life, 400 nmol 1 week later, and 800 nmol at 15 d of age. Control animals were given 5, 10 and 20 μl of DMSO using the above schedule. The newborn mouse is very sensitive to i.p. injections, and many animals died within 2 weeks following injection with the control vehicle or with the various compounds on days 1, 8 and 15 of life. The animals were weaned at 25 d of age. Large differences in survival among animals treated with DMSO, BP or BP 7,8-dihydrodiol did not occur until after the animals were 25 d old (Fig. 1). The experiments were ended 21-24 weeks after their initiation. After the initial deaths in all groups during the first 2 weeks of age, most of the remaining BP 7,8-dihydrodiol-treated animals died by 24 weeks of age, whereas most of the BP- and DMSO-treated animals that survived until weaning were still alive. Complete histopathological examination was performed on most animals (see Table 1 legend). The examination included routine study of the hematopoietic tissues and counting of macroscopic pulmonary adenomas. Seventy per cent of the BP 7,8-dihydrodiol-treated animals thus examined developed malignant lymphoma (leukaemia), which was apparently a major cause of death of these animals (Table 1). Almost all cases of malignant lymphoma were generalised with involvement of the thymus, spleen, bone marrow, lymph nodes, liver and other organs. Of special interest was the very short latency period for the development of malignant lymphoma. The first diagnosis of malignant lymphoma was made in an animal treated with BP 7,8-dihydrodiol, which died at 5 weeks of age. No cases of malignant lymphoma were recorded among the BP- and DMSO-treated animals. BP 7,8-dihydrodiol also induced pulmonary adenomas. Although the first pulmonary adenoma was found at an early age (14 weeks), induction of this type of tumour was slower than that of the malignant lymphomas. Among the BP 7,8-

dihydrodiol-treated animals which died and were autopsied between 4 and 17 weeks of age, 31% had lung adenomas, and 65% had malignant lymphomas. Among the BP 7,8-dihydrodiol-treated animals which died or were killed at the age of 18–24 weeks, 92% had lung adenomas and 75% had malignant lymphomas. The BP-treated animals also developed lung adenomas, but the number of pulmonary adenomas per animal was much lower when compared to BP 7,8-dihydrodiol-treated animals (Table 2). Only 4% of the DMSO-treated animals developed pulmonary adenomas. Three animals treated with BP 7,8-dihydrodiol developed a total of two skin papillomas and one skin fibroma. Fibroma of the skin was also found in two of the BP-treated animals. There were no significant differences in death rate or tumour incidence between male and female animals.

Table 2 Pulmonary adenomas in newborn mice treated with BP, BP 7,8-dihydrodiol and diol epoxide-2

Treatment	Total dose (nmol)	Sex	No. of animals	Adenomas/animal (mean $\pm$ s.e.)
Control	0	♂	32	<0.1
		♀	16	0
		Total	48	<0.1
BP	1400	♂	24	5.1 $\pm$ 1.6
		♀	20	7.7 $\pm$ 1.3
		Total	44	6.3 $\pm$ 1.0
BP 7,8-dihydrodiol	1400	♂	8	77 $\pm$ 16
		♀	6	72 $\pm$ 27
		Total	14	75 $\pm$ 14
Diol epoxide-2	28	♂	17	2.8 $\pm$ 1.0
		♀	20	6.8 $\pm$ 1.1
		Total	37	4.9 $\pm$ 0.8

Swiss-Webster mice [BLU:Ha (ICR)] were injected with BP and BP derivatives (Fig. 1, Table 1). Animals that were killed at the end of the study, at 21–24 weeks of age, were used for determination of the number of adenomas per lung.

In a preliminary experiment, newborn mice were injected separately with each of the two diastereomers of BP 7,8-diol-9,10-epoxide (diol epoxide-1 and diol epoxide-2). These compounds were very toxic, and 80–90% of the animals died within 48 h after a single injection of 200 nmol of diol epoxide-1 or -2. A reasonable survival of animals was achieved only after decreasing the dose of these diol epoxides to 2% of the dose of BP or BP 7,8-dihydrodiol. Four nmol of diol epoxide-1 or -2 was given on d 1 of life, 8 nmol on d 8 and 16 nmol on d 15. However, this dose of diol epoxide was still more toxic to the animals than a 50-fold higher dose of BP or BP 7,8-dihydrodiol (Fig. 1, experiment 2). The experiment was ended when the mice were 21 weeks old, and lung adenomas were found in 81% of the animals treated with diol epoxide-2 (Table 1). For diol epoxide-2-treated animals which had survived until weaning, about the same incidence of pulmonary adenomas had occurred as with a 50-fold higher dose of BP (Table 2). Diol epoxide-2 did not induce malignant lymphomas in the newborn mice. Diol epoxide-1 was more toxic than diol epoxide-2 in the newborn mice (Fig. 1 and unpublished observations), and there were not enough survivors available for determining the carcinogenicity of diol epoxide-1. Additional studies are now in progress to determine the comparative carcinogenicity of these two diol epoxides of BP.

Studies on the carcinogenicity of BP metabolites on mouse skin showed that BP 7,8-oxide was carcinogenic¹⁹ and that BP 7,8-dihydrodiol was a more potent carcinogen than BP 7,8-oxide (ref. 20). H₄-BP 7,8-epoxide, however, which is related to BP 7,8-oxide but lacks the double bond in the 9,10-positions of the molecule, did not show any carcinogenic effect, suggesting that a BP 7,8-diol-9,10-epoxide might indeed be an ultimate carcinogenic metabolite of BP. Since these earlier studies with BP 7,8-dihydrodiol and BP were performed with doses which were at the top of the dose-response curves, no conclusion was reached as to whether BP

7,8-dihydrodiol was more carcinogenic than BP. Our results here indicate that BP 7,8-dihydrodiol is a more potent carcinogen than BP when injected into newborn mice and is therefore a proximate carcinogenic metabolite of BP. Diol epoxide-2, a very potent mutagen derived from BP^{10–12}, has the same carcinogenicity as BP at a 50-fold lower dose in newborn mice. The extremely high carcinogenic activity of diol epoxide-2 suggests that it is an ultimate carcinogenic metabolite of BP.

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matters arising

Lunar and meteoritic palaeomagnetism: common origin contested

BANERJEE and Mellema^{1, 2} (BM) have suggested that both the lunar crust and the primitive carbonaceous chondrites (CC) have been magnetised simultaneously by strong ($\lesssim 1$ Oe) magnetic fields associated with an early T-Tauri active phase of the Sun. Here I argue that this conclusion is untenable. Their basic premises are, first, similar ages of, and, second, comparable palaeofield strengths for, lunar rocks and CC.

The CC and their constituents are among the oldest solar system objects dated thus far³⁻⁷ ($\sim 4.6 \times 10^9$ yr). Sensitive ¹²⁹I chronometry revealed sharp isochronism for CC magnetite forma-

fields¹⁰, could account well for several observed magnetic features of CC¹¹. Moreover, recent results on the electrical properties of CC¹² seem to link their magnetic and thermal history satisfactorily in the framework of solar-wind induction heating of asteroids with carbonaceous surfaces, which at present dominate by number in the main-belt¹³. Observations of young active stars¹⁴ and calculations of 'T-Tauri lifetimes' for $\sim 1M_{\odot}$ stars^{15,16} show that an epoch of intense mass loss for an evolving Sun could have lasted 1–10 Myr. This is compatible with the above chronology of CC, but raises a major difficulty for BM in the case of lunar rocks.

First, the early chronology of lunar crust differentiation cannot be similarly established due to an early intense bombardment, which lasted up to $\sim 3.9 \times 10^9$

of the ancient lunar crust¹⁷. Furthermore, a strong case was recently made for excluding any external source field for globally magnetising the ancient lunar crust^{18, 19}, so that the BM suggestion is weakened on both theoretical and observational grounds.

The claim that comparable paleo-field estimates of $\lesssim 1$ Oe for lunar rocks and CC should be taken at face value can also be challenged. Before discussing palaeointensities we must be concerned with the presence of a demonstrably ancient NRM component. CC evidence looks quite convincing^{11,20-22}, but it is still doubtful that any lunar rocks have preserved an indigenous palaeoremanence^{23, 24}. The mechanism by which this NRM was imprinted is also at issue: the CC are compositionally the most primitive and thermally the least altered ex-

Table 1 Palaeofield intensities from carbonaceous chondrites

Meteorite (type, subtype)	Palaeofield (Oe)	pTRM ($T^{\circ}\text{C}$, H Oe)	NRM fraction matched	NRM type	Ref.
Orgueil (I, C1)	$\begin{cases} 0.67 \\ 0.02 \end{cases}$	$\begin{cases} (120, 0.5) \\ (250-120, 0.5) \end{cases}$	$\begin{cases} T_B < 73^{\circ}\text{C} \\ 120^{\circ} < T_B < 250^{\circ}\text{C} \end{cases}$	$\begin{cases} \text{TRM} \\ \text{CRM} \end{cases}$	$\begin{cases} 21 \\ 21 \end{cases}$
Ivuna (I, C1)	0.5	—	Whole coercivity spectrum	(CRM+DRM)	11
Murray (II, C2)	0.7 (0.33–1.35)	(250, 0.5)	$H_{AF} > 100$ Oe	Non-thermal	11
Murchison (II, C2)	$\begin{cases} 0.4 \\ < 3 \end{cases}$ $\begin{cases} 0.18 (0.17-0.19) \\ 0.01-0.02 \end{cases}$	$\begin{cases} (250, 0.5) \\ (90, 0.5) \\ (90-250, 0.5) \end{cases}$	$\begin{cases} 100 < H_{AF} < 300 \text{ Oe} \\ H_{AF} > 300 \text{ Oe} \\ T_B < 90^{\circ}\text{C} \\ 90^{\circ} < T_B < 150^{\circ}\text{C} \end{cases}$	$\begin{cases} \text{Non-thermal} \\ \text{pTRM?} \\ \text{TRM or CRM} \end{cases}$	$\begin{cases} 11 \\ 21 \end{cases}$
Renazzo (II, C2)	2.3	(250, 0.5)	All	pTRM or TCRM	11
Allende (III–V, C4)	$\begin{cases} 0.95 \\ 0.25 \\ 0.15 \end{cases}$ 1.09(0.96–1.23) 1.1(0.93–1.3)	$\begin{cases} (150, 0.5) \\ (350, 0.05) \\ (110-130, 0.5) \\ (150, 0.59) \end{cases}$	$\begin{cases} H_{AF} < 500 \text{ Oe} \\ H_{AF} > 500 \text{ Oe} \\ 400 < H_{AF} < 600 \\ 20^{\circ} T_B < 130^{\circ}\text{C} \\ 30 < T_B < 150^{\circ}\text{C} \end{cases}$	$\begin{cases} \text{Non-thermal} \\ \text{pTRM} \\ \text{Non-thermal} \\ \text{TRM} \\ \text{TRM, TCRM} \end{cases}$	$\begin{cases} 11 \\ 21 \\ 22 \end{cases}$

H_{AF} ≤ peak alternating field value which gives microcoercivity of remanence; T_B , blocking temperature; (p) TRM, (partial) thermoremanent magnetisation; TCRM, thermochemical remanence; CRM, chemical remanence; DRM, depositional remanence.

tion (within 2×10^5 yr) and indicated rapid aggregation and brief metamorphism, lasting at most 1 Myr (ref. 5). An upper limit on the duration of a precompaction irradiation episode of CC component grains (by solar wind and solar flares) was recently estimated at < 4.5 Myr, but typically $\lesssim 1$ Myr, at nominal heliocentric distances of 10 AU (ref. 8). These and the enrichment of solar-wind implanted gases in CC magnetite⁹ strongly support my earlier suggestion that an enhanced early solar wind, convecting strong magnetic

yr ago and led to its extensive brecciation and metamorphism¹⁷. Second, since BM assume implicitly that lunar rocks carry a thermoremanence (TRM) acquired at cooling in an external magnetic field, it is reasonable to date it by the crystallisation ages of lunar basalts and breccias, which range from ~ 3.3 to $\sim 4 \times 10^9$ yr (ref. 17). This requires an excessively long ($0.6-1.3 \times 10^9$ yr) active phase for maintenance of strong fields. Also, it is unlikely that an older magnetisation (4.6×10^9 yr) has survived the violent impact metamorphism

traterrestrial samples known. Their NRM is admittedly a low temperature magnetisation, although detailed interpretations differ^{11,21,22} (Table 1). In contrast, most lunar rocks for which palaeointensities have been obtained are thermally and mechanically modified by impacts.

It is even doubtful whether lunar rocks are capable of acquiring TRM in the conventional sense^{26, 27}.

In this light, the fact that entirely different experimental techniques have yielded similar values for the ancient

field strength (~ 1 Oe) in several CC^{11, 20} (Table 1) inspires confidence in their validity. In contrast, the lunar palaeointensity determinations are notoriously controversial^{23, 25–27}. Some of the new techniques used (refs 25, 28, 29) are not yet established even for terrestrial rocks, whose magnetic mineralogy is more familiar and better understood. Banerjee and coworkers fuelled the debate with 'reliable' palaeofield estimates of ~ 0.01 Oe for three Apollo 15 basalts^{28, 29}, including one (15535) which they had earlier shown to contain no useful remanence at all^{30, 31}. Their later work on an Apollo 17 foliated breccia boulder produced no less than three distinct sets of paleointensities ($0.18\text{--}0.74$ Oe)^{32, 33}, differing significantly both within and between adjacent foliation layers. These and divergent directions of 'stable' palaeoremanence forced the conclusion that the lunar magnetising field must have changed considerably both its direction and its intensity during the assembly of this 2-m boulder 4.0×10^9 yr ago. It seems more plausible to argue that the magnetic data are not internally consistent, or to ascribe the magnetic behaviour of breccias to textural effects of compaction shock²⁴.

Another corollary to the BM hypothesis is that similar palaeofield strengths imply a common source for lunar rocks and CC. Such reasoning could lead to the fallacious conclusion that the oldest (3.85×10^9 yr) terrestrial rocks were also magnetised by extended solar wind fields. Within the BM context, comparable magnetic field strengths imply that the Moon and the carbonaceous meteorites condensed and accreted at the same heliocentric distance, in contradiction to most current chemical nebular models³⁴. If the CC have originated in the asteroid belt¹³ (3–5 AU), whereas the Moon accreted near 1 AU, the present radial dependence of the solar wind magnetic fields would require at least 3–5 Oe for a tightly wound spiral configuration, but up to 10–25 Oe for radial flow. These values are high even by the currently inflated lunar-magnetic yardstick.

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BANERJEE REPLIES—There was one major point that we had wanted to make in our original communication¹. Before, and even since the publication of our note, there have been no lunar palaeointensity experiments in which, first, a direct, thermal method had been used on three sub-samples from one rock and,

second, magnetic monitoring by Anyhysteretic Remanent Magnetization (ARM) had been carried out on the samples before and after the heating. In spite of the well-known magnetic variability of lunar samples, as underlined by Dr Brecher², what was amazing to us was that our three samples were magnetised homogeneously enough to yield an average palaeointensity of 0.4 Oe with a s.d. of about 40%. In contrast, in the lunar palaeointensity measurements, the highest reliability that one usually hopes to achieve is the order of magnitude of the field!

Our second point was that if the palaeointensity was indeed of the order of 0.4 Oe at about 4.0×10^9 yr (the average radiometric age of the rock), and if the field was internally generated from the Moon, the Sonnett and Run-corn³ dynamo in a small, early lunar iron core could be the only plausible internal source. We then drew attention to the similarity of the size of this field to that postulated earlier for carbonaceous chondrites (age $\sim 4.4 \times 10^9$ yr) by us⁴, and by Dr Brecher⁵. I agree wholeheartedly with her that "the fact that entirely different experimental techniques have yielded similar values for the ancient field strength (~ 1 Oe) in several CC (carbonaceous chondrites) inspires confidence in their validity. In contrast, the lunar palaeointensity determinations are notoriously controversial". But, we have done our best in searching for better methods, used multiple sub-samples and published the details of our technique⁶. Our interpretation, and that of any other groups of scientists, must necessarily be a function of our knowledge at the time of writing. None of the references quoted by Brecher can convince anyone that "it seems more plausible to argue that the magnetic data (that is, the data of refs 1 and 6) are not internally consistent", specifically when such a search for internal consistency of palaeointensities has not been undertaken.

Our hypothesis that both the meteorites and the ancient lunar samples could have been magnetised by external solar fields is admitted by the fact that the upper limit T-Tauri phase solar wind pressure would correspond to a magnetic pressure of the order of a few Oe at 1 AU. But, as we pointed out, whether this was actually so cannot be proved without more extensive and reliable data on both meteorites and lunar samples. We had also suggested that the apparently large (0.4 Oe) field could be the result of a smaller ambient solar field, magnetohydrodynamically amplified by plasma dynamos operating on the lunar crust during the intense collisioning stage, at and before 4.0×10^9 yr. I am sure there are others who could think up more alternative explanations. The purpose of our communication was to

alert other workers about the existence of our data and to encourage them to think of plausible models.

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Digynic triploidy after superovulation

DIGYNIC triploidy after superovulation in mice reported by Takagi and Sasaki¹ may be closely related to the aging of eggs at fertilisation rather than superovulation through the administration of exogenous gonadotrophins. It is well known that the fertile life of eggs after ovulation is about 10 h and that ageing of eggs results in a decrease of fertilisation rate, an increased incidence of abnormal fertilisation and subsequent degeneration of embryos. Suppression of the second polar body formation resulting in digynic fertilisation occurs when eggs age in the

hamster², mouse³ and other species⁴. Since the ovulation of a large number of eggs is not expected to occur simultaneously and the time of ovulation for each individual egg would be more spread out during superovulation than after normal ovulation, the chances of delayed fertilisation and digynic triploidy would be higher after superovulation than normal ovulation. Most of the fertilised eggs from superovulated rabbits, however, can develop into normal young after transferring them to several recipient rabbits⁵.

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Nitrate reductase as predictive test of crop yield

THE report of Johnson, Whittington and Blackwood¹ on using nitrate reductase as a predictive test of crop yield is probably valid but there are several problems with both the *in vitro* and *in vivo* assay they used which deserve attention.

The maximal *in vitro* activity shown in Fig. 1 of ref. 1 is about 0.1 $\mu\text{mol g}^{-1} \text{h}^{-1}$, a value considerably lower than the activities (~ 10 – $12 \mu\text{mol g}^{-1} \text{h}^{-1}$) commonly observed in leaves of wheat^{2–4}. The disparity is so large as to cause concern about the assay procedure. Although the latter activities were obtained without casein in the extraction medium it is conceivable that the inordinately low activities obtained by Johnson *et al.* were partially due to failure to protect nitrate reductase from a constitutive serine protease which rapidly degrades nitrate reductase *in vitro*^{2,5,6}. In *Escherichia coli* a serine protease acts to remove nitrate reductase from membranes⁷ and in higher plants this may be a reason why its half life is in the order of 2–4.2 h (refs 8, 9). Incorporation of casein or phenylmethylsulphonylfluoride (PMSF) into the extraction media^{5,6} obviates this protease activity and yields activities approximately 100 times^{10,11} those reported by Johnson *et al.* Therefore, if *in vitro* nitrate reductase activity is to be used as a predictive assay, it should be assayed as described above to avoid complications resulting from

differential degradation during extraction.

The problem with the *in vivo* assay of nitrate reductase is also serious. This assay may or may not be proportional to the *in vitro* activity^{12,13}, but typically represents only about 20% of the *in vitro* activity¹². Also, the electron donor for the *in vivo* assay has never been identified, nor is it clear that the electron donor *in situ* is identical to that which serves in the conditions employed in the *in vivo* assay. Therefore, it seems unwise to use this assay to estimate any parameter associated with nitrate assimilation unit it is established exactly what the *in vivo* assay is measuring.

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JOHNSON *et al.* REPLY—While a number of interesting points are raised by Butz, it is difficult to see why his criticisms are directed specifically at our work. In any study where enzyme activities are measured either *in vitro* or by the so-called *in vivo* assays it is difficult to state categorically whether or not the measured rates reflect accurately the true activities of the enzymes within the cells. The essential point surely is that our experiments¹ and in the many experiments from Hageman's group in Illinois^{2,3}, a correlation has been shown between nitrate reductase activity as measured, and the yield of a crop. For such a correlation to be valid or useful the precise biochemical basis is not necessarily important. In this case, however, the correlation does provide valuable evidence that the enzyme activity measured is related to the process of nitrogen metabolism.

Some of the points raised by Butz do require some further comment. First, the references to the work of Schrader *et al.*^{4,5} are misleading. In their investigations the improvement in nitrate reductase activity obtained

using casein was strongly dependent on the type and age of the tissue employed. With the youngest leaf tissue (similar to that used in our study) the improvement was only 10% compared with the 100-fold quoted. This latter value was obtained only with old tissue of one particular variety of maize and cannot be considered in any way typical. In our investigation some of the *in vitro* wheat nitrate reductase assays were duplicated with *in vivo* assays. These gave substantially similar results. Casein is nevertheless a useful protector of nitrate reductase *in vitro*, although it is by no means the most effective one in all tissues.

Second, Butz speculates that the protease which attacks nitrate reductase does so by removing the enzyme from membranes. This seems unlikely as neither in *E. coli* nor, judged by the majority of the available evidence⁶, in higher plants is assimilatory nitrate reductase membrane bound.

Third, in view of the problems known to be associated with the *in vitro* assay of nitrate reductase, of which those referred to by Butz are by no means all, it seems to us unwise to criticise an *in vivo* merely because it sometimes produces results differing from those obtained with an *in vitro* procedure. The fact that the electron donor is not known does not seem important in this context. Recent reports of differences between results obtained using the two procedures may have obscured the fact that in the majority of cases very similar results are obtained with both methods. For example, Hageman's group³ showed a correlation of 0.99 between *in vitro* and *in vivo* assays of nitrate reductase in several varieties of wheat. Furthermore, a good correlation was obtained between nitrate reductase activity and nitrogen assimilation, thus confirming our observations.

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Control of feline leukaemia virus

HARDY *et al.*¹ report the success of a programme for preventing spread of feline leukaemia virus (FeLV) among pet cats by screening for FeLV viraemic individuals, removal of carriers and quarantine of contacts. Although evidence that the programme was of some success seems strong, the study's methodology deserves comment. Major problems concern the comparisons between 'treated' and 'untreated' groups.

(1) The groups were 'self-selected' by owners. Though it is probable that pet owners' responses to such a programme reflect different approaches to the care of pets, we are given no information on the cluster-size distribution, location, management, sex or age structures of the groups—though each such variable has major implications for the spread of an infectious agent.

(2) The only information given as to the previous status of the groups is that the treated group had a lower initial prevalence rate of FeLV carriers than did the untreated control (0.224–0.312). This indicates that the treated group was at a lower risk of infection even before the programme and thus it is improper to attribute subsequent differences in incidence rates to the programme alone.

(3) The report of a 0.193 incidence rate in the untreated group over three months is surprising, as it implies an annual incidence rate of 1.0—(1.0–0.193)⁴ or 0.576, which is far higher than the prevalence rate in either group—though the authors claim that most viraemic cats remain viraemic for life, they report a low natural mortality rate for viraemic individuals and they make no reference to reversions to virus negative among the untreated group cats.

(4) The programme entailed both removal of viraemic individuals and also quarantine of contacts. As neither action was imposed upon the untreated group it is improper to attribute the lowered incidence rate to removal of FeLV carriers alone.

(5) Mortality 'rates' are given without reference to time period or to background mortality among 'uninfected' cats. Without such references they are uninterpretable.

(6) The authors' conclusion that infection in most cats occurred between 18 and 24 months of age does not follow logically from their data on median ages—and, if it did, it would emphasise the necessity to adjust for age when comparing incidence and

mortality rates. No such adjustments have been made.

The implications of Hardy *et al.*'s study are important. Though some of the problems mentioned here may have arisen due to the demands of brevity in publication, it should be stressed that such studies deserve a careful methodology. This is especially true in the context of current interest in the epidemiological implications of oncornaviruses.

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HARDY AND McCLELLAND REPLY—We agree with Dr Fine that a 42-fold decrease in feline leukaemia virus infection (FeLV) in the households which implemented our FeLV test and removal programme is evidence that our programme was successful¹. Our study was not a strict epidemiological survey, indeed, an epidemiological study of lymphosarcoma in cats failed to detect the contagious nature of the disease². But, we would like to answer Dr Fine point by point.

Matters Arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 300 words and the briefest of replies, to the effect that a point is taken, should be considered.

(1) All the owners were given the same advice regardless of whether or not they chose to have their infected cats removed. The management of the households was the same for both groups and the average number of cats per household (Fine definition of cluster) in those households in which the cats were removed was 16.6 cats per household while in the other group it was 16.5 cats per household (Table 2, ref. 1). Households in both groups were located throughout the USA. The two groups are thus quite comparable.

(2) Although it is true that the initial FeLV infection rate of the two groups was different, the difference in

the rates of FeLV infection between the two household groups after our programme had been implemented was much greater and was statistically highly significant ($P < 0.001$).

(3) Dr Fine should not assume that the FeLV infection rate of 19.3% in the first 3 months will be perpetuated. The infection rate decreases because, as we reported, approximately 41% of the exposed cats in a household develop protective FeLV neutralising antibody titres though they never test positive for FeLV. We reported previously that 33% of healthy exposed cats became FeLV infected³. This figure was the result of a single sampling of many households in which the cats had been exposed to FeLV for varying periods of time. In some households, 100% of the cats were infected, while in others only 10% were infected, which shows that the FeLV infection rate can vary greatly. In contrast to Dr Fine we do not think that our observed mortality rate of 52% for secondarily infected cats and 8.5% for first test FeLV uninfected cats is low. With regard to his other points in this area; most cats do remain viraemic for life and reversions happened only rarely among the cats in this study.

(4) Both groups of households were quarantined as referred to in refs 13 and 16.

(5) The observation period for the study was 2 y, as mentioned in the paper¹.

(6) The median age of cats which became infected with FeLV was simply our observation on 49 cats—we did not make any conclusions about age susceptibility of pet cats in the general population.

In conclusion, we would like to add that removal of the carrier host is a common method for controlling and preventing the spread of many infectious veterinary diseases when vaccination is not available. For instance, bovine tuberculosis, brucellosis, foot and mouth disease, hog cholera, African swine fever, vesicular exanthema, rinderpest and glanders are controlled by test and removal programmes. Thus, the concept of preventing the spread of the contagious feline leukaemia virus by a similar programme should not, we think, be difficult to accept.

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reviews

Entering the genetic arena

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Who Do You Think You Are? Man or Superman: The Genetic Controversy. By Oliver Gillie. Pp. 255. (Hart-David, MacGibbon: London, 1976.) £4.95.

OF LATE, genetics, and particularly human genetics, has found itself increasingly involved in controversial social issues; these include the roles of heredity and environment in the origin of the apparent differences in performance on intelligence tests, and the relationship, if any, of an extra Y chromosome to criminality. The scientific discussion of these issues has been characterised by more intellectual arrogance and humbug than seems generally to prevail. I had hoped that this little volume might redress these excesses in a trenchant way. It does not. *Who do you think you are?* has a catchy title, a few provocative thoughts, but is generally disappointing. We are confronted with clichés, and an overly pejorative division of the world into hereditarians and environmentalists. The attributes imputed to these groups would be worthy of a medieval scholastic obliged to account for the presence of evil in a world produced by a deity intrinsically good. What of value can possibly come from further polarisation of the attitudes toward these problems? These are strong, possibly intemperate remarks to some. Let me document them.

We find on p14 such simple clichés about political beliefs as, "‘Liberal’ or ‘radical’ scientists tend to be ‘environmentalists’ and ‘conservative’ scientists tend to be ‘hereditarians’ who believe that heredity is of preeminent importance in deciding character. This is exactly what we might expect when we reflect that it is conservatives who always have the greatest interest in property and titles—that is, the greatest vested interest in non-biological inheritance." Or on pp109–110 we are treated to an analysis of Johannes Lange's personal prejudices and how the latter coloured not only the data on criminality he collected but his interpretations of these data as well. These accusations may indeed be true, but the cast of the argument seems to indict all "hereditarians" but exonerate "environmentalists" of this not so admirable human failing. One wonders

whose prejudices are showing here. The larger issue, or so it seems to me, is not whether Lange or others exhibit prejudices but what is the sequence of events or experiences, cultural or biological, which gives rise to them. Self-serving irrelevancies are everywhere. The late Trofim Lysenko is described as being "from a simple peasant background" (p24); arguments which the author favours are prefaced with remarks such as "few serious scientists now dispute" (p128)—the implication is clear. Darlington's *Genetics and Man* doubtlessly deserves the criticisms which Gillie levels, but who other than he has ever characterised *Genetics and Man* as a "major work on the biology of man" (p95)?

There are, moreover, simple mistakes. DNA is said to have been discovered in 1953 (p20); this is the year in which its structural form was first identified but its chemical composition had been known for almost a century at that time. The inheritance of aryl hydrocarbon hydroxylase as here described (p129), has not been confirmed although the relationship of the inducibility of this enzyme to lung cancer seems still to hold. Although the book is generally well produced from the printer's vantage point, there are a

few ludicrous misprints. I'll cite one. The dust jacket identifies William Shockley as Schockley, which in view of the racism of which he is charged, or at best the naïve succour of racists ascribed to him, borders on shtick.

I cannot urge the reader of this review to dash out and purchase *Who Do You Think You Are?* for unless he or she belongs to the uncertain audience to which it is addressed he (or she) will be disappointed. As I attempted to imply at the outset, the book could have been more, and Oliver Gillie could have written one which would serve our time importantly. I fear, however, he has pandered where he should have been principled; has been simplistic where he should have been simple; has polarised where he should have been understanding; and has generally failed to appreciate the power of satire, if his intent is to serve a particular cause or point of view. I would urge, as a model, he read (or re-read) Haldane's *Politics and Heredity*, an opus of the 30s, before he ventures into this arena again. □

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Nucleic acid treatise

Photochemistry and Photobiology of Nucleic Acids. Edited by Shih Yi Wang. Vol. 1: Chemistry. Pp. xv+576. (Academic: New York and London, 1976.) \$54; £29.50.

THIS is the first of a two-volume treatise the purpose of which is to summarise our present knowledge of the photochemistry and photobiology of nucleic acids. The present first volume deals with the physical and chemical processes induced by absorption of a photon in nucleic bases—including rare and synthetic ones—as well as their monomeric and polymeric derivatives. The second volume is devoted to the DNA photochemistry and the photobiological aspects.

Each chapter is written by a group of workers, who are specialists in their respective fields. The basic concepts in

the domain of photochemistry are first outlined, followed by a complete and up-to-date review on the excited states of nucleic acids. Emphasis is, of course, placed on the new and exciting developments concerning room temperature emission.

The main core of the volume is the description of those photoproducts known so far. Particularly pertinent is the analysis of the photohydrates and of the cyclobutane-type photodimers. The volume succeeds in presenting a balanced point of view on conflicting data or interpretations coming from different groups, and discloses to the reader the problems that remain to be resolved.

Interesting chapters are devoted to the other pyrimidine adducts, the list of which is rapidly expanding, and to the purine photochemistry. In particu-

lar, the spectroscopic and photochemical properties of nucleic acids in frozen aqueous solution are reviewed. Finally, there are three chapters concerned with the methodology which has proved to be so useful in establishing the chemical structure and detailed conformation of the photoproducts—mass and nuclear magnetic resonance spectrometry, and X-ray crystallography. Also included is a chapter on radiation chemistry.

The publication of this book is particularly opportune, coming at a time when the repair mechanisms of nucleic acids are so extensively studied. It

should therefore serve as an important source of references. Some aspects are redundantly treated, and some others of peripheral interest to the authors are briefly and in a few cases inaccurately mentioned. More fundamentally I would have expected a more integrated presentation of the chemical and biological data for a book intended to be a classical treatise.

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View from space

Earth Sciences in the Age of the Satellite. By J. Pouquet. Pp. ix+169. (D. Reidel: Dordrecht and Boston, Massachusetts, 1976.) Dfl.58; \$22.50.

THE view from space of the Earth's surface is providing a distinctive new source of environmental information. This distinctiveness is based firstly on the overview of large areas which makes possible the detection of large scale features that cannot be easily recognised either on the ground or from aircraft, and secondly on the possibility of monitoring rapidly changing phenomena using the regular repetitive imagery obtained from many satellites. Although imagery from satellites has been available for some years, many earth scientists have been rightly sceptical of (perhaps because of) the over-optimistic claims made for remote sensing. The more cautious, more carefully considered study of remote sensing that is now becoming increasingly common is leading many earth scientists to examine its potential, not as a panacea, but as one of several alternative data sources.

Earth Sciences in the Age of the Satellite, was first published in 1971, and has more recently been published in an updated form in this translation. This may well account for many of the naive hopes expressed about the role of remote sensing and satellites.

In the first of the book's three parts, properties of radiation from various parts of the electromagnetic spectrum and their interaction with the Earth and atmosphere are briefly described. The second part on the role of satellites is out of date, although it is a very useful compilation of information about the earlier satellites. The final part concentrates on the applications to the earth sciences, with greatest emphasis being placed on geopedological examples (*sic*). Many of the examples are superficial or even trivial and will convince few that remote sensing has any substantial potential. No proper evaluation of the remote sensing methods

is presented, nor is there any sensible objective comparison of their merits relative to other more conventional data gathering methods. In this and previous sections, the illustrations are often unhelpful and more and better photographic reproductions are clearly needed.

The idiosyncratic style of writing will be annoying to most readers and does not merely arise as a result of poor translation. Finally, the book is costly (£14.50) considering its length (169 pages), and fortunately there are other much better books now available.

J. R. G. Townshend

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Infrared and Raman spectroscopy

Infrared and Raman Spectroscopy. Part A. Edited by E. G. Brame and J. Grasselli. Pp. ix+345. (Dekker: New York and Basel, 1976.)

THIS is the first of a projected set of three volumes on infrared and Raman spectroscopy. The set is intended to cover all possible applications of these techniques in such diverse fields as the food industry, petroleum, textiles, biology, environmental science, and so on. This, the first volume, is more concerned with academic, rather than with industrial uses, but a sound understanding of the basic principles is obviously necessary before these techniques can be properly applied.

The first chapter is an introduction to molecular vibrations by Crawford and Swanson. It introduces the basic concepts in a manner suitable for those new to this field, with all the clarity and good humour one would expect from such a source. The chapter covers the origins of infrared and Raman spectra, and the experimental techniques, and introduces such concepts as selection rules, coupled oscillators, group frequencies, intensities and quantitative analysis.

The second chapter by Carter deals with inorganic materials. This delves more deeply into group theory, symmetry, and the selection rules, and shows in detail how the infrared and Raman spectra can be used to derive the structures of some specific inorganic molecules. It deals also with site-and-factor group analysis and with the spectra of oriented crystals. It concludes with some useful comments on experimental methods and with some character and correlation tables.

Chapter three, by Edgell, covers the vibrational analysis of organometallics. It extends the discussion of the selection rules to cover the concept of hidden symmetry, and again illustrates the application of the technique to a number of specific molecules. Finally, it discusses the derivation and significance of force constants in molecules of this kind.

Chapter Four, also by Edgell, is on ionic organometallic compounds. This deals almost entirely with a relatively new field, as the bands arising from the translational modes of ions in solution were not observed until 1965. The author shows that the study of the translational and intramolecular vibration bands can yield some very valuable information on the structure of solutions, on the motions in them and on the nature of the short range forces involved. This is a very highly specialised topic and includes some previously unpublished research by the author. Nevertheless it is a topic which is very properly included if the whole work is to meet its stated objective of covering all possible applications.

It is all too common to find that texts written by a number of different authors contain a good deal of overlap and repetition. It is therefore a pleasure to be able to report that this is not so in this case. The editors have done an excellent job of integration so that topics such as the selection rules are introduced in a preliminary way in the first chapter, carried further in the second and yet further in the third. The text is well presented, the diagrams are clear and the standards of proof reading have been high. I could detect only one error, the misplacing of DCN in Fig. 4.

New entrants to this field will find the first chapter comfortably simple and non-mathematical, but they must be prepared for increasing complexity as the book proceeds. This is however, a necessary requirement for the academic topics covered and they should not feel deterred from exploring the future volumes if their interests are primarily in industrial applications. Altogether this is an excellent start to what promises to be a very good and authoritative series.

L. J. Bellamy

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Thermodynamics and surface chemistry

Equilibrium in Solutions; Surface and Colloid Chemistry. By G. Scatchard. Pp. xxxv+306. (Harvard University: Cambridge, Massachusetts and London, 1976.) £20.40.

THE foundations of theoretical thermodynamics laid by Willard Gibbs represent a truly great intellectual and artistic achievement, perhaps marred a little for most of us by the feeling that it seems to require almost as much effort to understand or use or teach thermodynamics as we imagine to have been required for the original creation. George Scatchard devoted a lifetime of effort to the subject, understood it better than anyone else, and used it for a series of definitive research papers on the thermodynamics of complex systems, often systems of biochemical interest. He also taught a graduate course on the thermodynamics of solutions at MIT and an undergraduate course on surface and colloid chemistry. This book contains the text of lecture notes for these courses, originally prepared around 1950 and made available to students in mimeographed form. They were corrected by Scatchard before his death in 1973 and published in the present form with the aid of a grant from the Commonwealth Fund. Walter Stockmayer is quoted in the preface as stating that publication of a text 20 years after it is written "implies that it is unique and in some measure timeless", an assessment with which this reviewer fully concurs.

The goal of the section on *Equilibrium in Solutions* reflects the attitudes of the author's research papers. It is to be meticulously correct in theoretical concepts and equations, and at the same time to be completely practical in the sense that the discussion always focuses on real experimental data and procedures. There is no patience with theoretical "ideas" that do not relate to experimental measurement; on the other hand, when measurements are *more precise* than theory, then the theory must be empirically extended and it is important that the extension does not violate physical laws in simple limiting situations.

All the chapters are in a highly compressed style, difficult to read unless one is already somewhat familiar with the subject. But it is very rewarding once one gets the hang of it because all the essentials are actually there; in fact they stand out with greater clarity than they generally do in more comprehensive texts.

Electrolyte solutions are treated in greater depth than other topics, and

the treatment covers ion transport as well as equilibrium properties. The problems inherent to a definition of single ion activities are discussed, as are the various ways of extending the Debye-Hückel limiting law to higher concentrations. On the other hand, there is no mention of polyelectrolytes. Chemical equilibria are treated with extreme brevity (little more than a definition) and the reader will find no mention of practical aspects of treating chemical equilibria, such as are involved in 'Scatchard plots'.

The *Surface and Colloid Chemistry* section has six chapters: Introduction; Surface tension and interfacial tension; Polycomponent field surfaces; Adsorption on solids; Physical properties of colloid solutions; Electric effects at surfaces and in colloid solutions. It is even more compressed than *Equilibrium in Solutions*, being only 67 pages long, but again the essentials are there, with the notable exception that micellisation and other self-association processes are not considered. There are no literature references, so that these notes need to be read together with more modern texts (the plural is used advisably here because at least two separate texts will be needed to cover the material).

In addition to the scientific texts, we are provided with an appreciative introduction by I. Herbert Scheinberg, an

autobiographical note, and a list of Scatchard's publications. The major portion of the autobiography consists of a brief explanation for every paper listed in the bibliography, as befits a man for whom work was clearly a labour of love. Because of his own deep involvement, he had no patience with superficial science, and he labels a number of his papers as "polemics", designed to expose shallow thinking and fallacy. He also used book reviews as a vehicle for criticism, and both the reviews and the polemics are useful reading because the fundamental core of a subject, that Scatchard was always striving to reach, sometimes emerges especially clearly in relation to a point of contention.

This volume will have strong appeal to all who love to shape equations into a description of the real world (in preference to pictures), for it is the record of a man with unique facility for doing so. It also should be read by anyone who ever has to teach thermodynamics or surface chemistry, for it cannot help but sharpen his insight and ability to transmit it.

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H. Mohr

Lectures on Structure and Significance of Science

Approx. 22 figures. Approx. 280 pages. 1977

Approx. DM 29,80; approx. US \$ 13.20

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In preparation

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Hans Mohr is a natural scientist with a deep-rooted interest in the nature of scientific thought and in the significance of science. His first book on this subject entitled „Wissenschaft und menschliche Existenz“ was written in German (2nd edition, 1970). The present text is based on a series of 15 lectures which the author delivered at the University of Massachusetts during the fall term of 1975. In the Prologue "Science and Responsibility", some basic problems are formulated in more detail. While the treatment of the "Scientific Method" can already claim a well-established tradition of scholarship, the responsibility of the scientist has up to now only rarely been used in the consideration of the phenomenon of science. The author does not equate philosophy of science with epistemology, nor does he follow the tendency of excluding anything from consideration which might raise moral problems. Rather, the lectures on "The Ethics of Science", "The Crisis of Science" and "Science and Values" are as essential for the book as the epistemological treatment of "The Scientific Approach". Another characteristic of the book is that some traditionally epistemological problems, such as empiricism and rationalism, are investigated from the point of view of scientific knowledge.



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Mammalian peripheral nervous system

The Peripheral Nerve. Edited by D. N. Landon. Pp. xii+836. (Chapman and Hall: London, 1976.) £25.

VARIOUS aspects of present knowledge about the mammalian peripheral nervous system are reviewed in this book, special emphasis being placed on fine structure, and on the nature of the relationships that exist between the neurone and its supporting cells, end-organs, and other surrounding tissues.

The first nine chapters deal with the functional morphology of the myelinated nerve fibre (D. N. Landon and S. Hall); unmyelinated nerve fibre (J. Ochoa); perineurium and connective tissue (F. N. Low); sensory ganglia (A. R. Lieberman); anterior horn motoneuron (S. Conradi); spinal and cranial nerve roots (H. J. Gamble); ganglia of the autonomic nervous system (G. Gabella); sensory terminals (L. H. Bannister); and motor end-plates (G. F. Gauthier). The remaining chapters review motor end-plate function (A. J. Buller); chemistry and structure of myelin (N. A. Gregson); histochemistry of peripheral nerves and nerve terminals (J. F. Hallpike); selected aspects of peripheral nerve pathology (G. Allt); and electrophysiological properties of peripheral nerve (J. J. B. Jack).

This comprehensive survey, with its extensive bibliographies and superb illustrations, will provide a valuable reference work for those engaged in research on the mammalian peripheral nervous system. Naturally, in a work of such length and scope and varied style some parts appeal more than others. For me the authoritative reviews on myelinated and unmyelinated axons, and on the nerve sheaths, are among the most interesting and useful contributions. There is also an excellent account of Wallerian degeneration and subsequent regeneration (excluding re-innervation), and an admirably comprehensive review of receptors.

The editor is to be congratulated for embarking on such a worthwhile enterprise and bringing it to a successful conclusion. There are signs, however, that active and critical editorship was sometimes lacking. For example, the consecutive chapters on motor end-plate structure and function should have been edited so that one complements the other. As it is, no functional comment is made on much of the ultrastructural information; "tonic" and

"slow" muscles in one are respectively "slow" and "slow-twitch" in the other; references to "Normanski" optics in the chapter on function remain uncorrected; and the use of the term "*en grappe*" is left in unnecessary confusion. Another contributor is allowed to depict a periaxial space in the Golgi tendon organ, and to present a bizarre account of muscle-spindle innervation in which, for example, the secondary sensory endings are supplied by "group IIa" afferents, and a collateral motor innervation is derived from axons belonging to "group IIb". The editor certainly knows better than this; perhaps it is his view that an editor should do little more than serve as the channel through which his sources flow freely and unfiltered from pen to print. At times that way can leave the reader floundering.

The book, over 800 pages long, is heavy (1.5 kg) to handle and at times heavy to digest. Paragraphs sometimes run on far too long and occasionally there is a rash of instructional italics. Publication evidently took three years, writing being mostly completed by 1973, although an effort has been made to update references to 1975. It might have been better to have published the work in two volumes; better still to have included other aspects and run to three. The value of a well-documented survey of this standard would certainly have warranted that.

David Barker

David Barker is Professor of Zoology at the University of Durham, UK.

Heat on the move

Thermal Conduction in Solids. (Oxford Studies in Physics.) By R. Berman. Pp. xi+193. (Clarendon: Oxford; Oxford University: London, December 1976.) £9.75.

EVERY now and again one happens gratefully on a monograph born purely of the author's desire to communicate, to distil the fruits of a lifetime's personal interest and research into a form which renders the topic accessible to a wider audience. This is just such a book. Our present relatively mature understanding of the mechanisms of thermal conduction in solids owes much to a steady succession of illuminating experiments carried out during the past thirty years by Dr Berman and his coworkers; so that, being a gifted expositor, he is particularly well fitted to convey to others some of his continuing fascination with this technologically important subject.

The book is aimed mainly at physics graduates (or, indeed, final-year under-

graduates) who want to progress beyond the rather limited discussion of thermal conduction usually provided by general textbooks on solid-state physics, but who lack the stamina necessary to tackle the unusually abstruse symbolism of the monumental work by J. M. Ziman (*Electrons and Phonons*, Clarendon, 1960). Throughout, Dr Berman's emphasis is very much towards conveying an understanding of the topic in question, and he has eschewed the algebraic pendants which, in the name of rigour, can so easily obscure the physics for most readers. The dustcover's description of his treatment as "detailed but uncomplicated" is entirely apt.

The book opens with an explanation of what the thermal conductivity coefficient is, how it is measured, and the general way in which it behaves for a variety of different materials. The next six chapters, dealing with conduction in non-metals, include discussions of phonons, phonon-phonon interactions, the scattering of phonons by defects, the Boltzmann equation and methods for solving it; and they explain how these concepts and techniques are able to account for the features of conduction observed in nearly perfect crystals, imperfect crystals, and amorphous materials. Of particular note are the sections which explain the subtle influence of normal (momentum-conserving) processes, Poiseuille flow of the phonon gas and a number of interesting resonant phonon-scattering mechanisms. These are topics which in most textbooks are dealt with either cursorily or not at all. The second half of the book deals lucidly with situations in which heat is transported by electrons, discussing the effect on the conductivity of various types of electron scattering in metals and alloys including superconductors, and in semiconducting materials.

The deliberate emphasis towards a description of work below room temperature is justified by the fact that such experiments usually provide a more sensitive test of the theory than is afforded by those carried out at higher temperatures. An extensive set of references to original papers is provided, and will enable readers to follow up particular aspects of earlier work which are of interest or of relevance to their own research. Books and review articles are also cited where they are likely to be helpful.

Dr Berman's well integrated and readable little monograph has filled what—with hindsight—was an obvious lacuna in the literature. It will continue to be of value for many years to come.

P. V. E. McClintock

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obituary

Sir David Martin

SIR DAVID CHRISTIE MARTIN, the Executive Secretary of the Royal Society, died suddenly on Thursday 16 December 1976, after nearly thirty years' service as the principal executive officer of the Society. Born on 7 October 1914 in Kirkcaldy (where he did an early morning paper round which inured him to a lifelong regimen of early rising), and starting from a local primary school he proceeded to Kirkcaldy High School and thence in 1933 to Edinburgh University. After first class honours in chemistry in 1937, he took a Ph.D. in 1939 under the supervision of J. A. V. Butler on organic compounds containing deuterium. His flair for administration had already shown itself: he was the first President of the Science Students' Union at King's Buildings, and he was much concerned in the Students' Representative Council and the Athletic Club. Originally intending to become a school teacher, but having had his horizons broadened by research and by student activities, he became in September 1939 Assistant Secretary of the Royal Society of Arts, which immediately released him for service in the Research Department of the Ministry of Supply for the duration of the war.

This last experience convinced him of the contribution that science could make to human affairs, even though he noted that altruism and an ability in science were not always linked characteristics in human make-up. His success in administration brought him to the notice of senior scientists, and resulted in his appointment in 1945 as General Secretary of the Chemical Society and in 1947—at the age of 32—as Assistant Secretary of the Royal Society. In 1943 he had married Jean MacGaradh Wilson; and throughout his life their home, first at Stanmore and then in the new premises of the Royal Society, was a centre of warm hospitality.

As chief executive of the Society, he found himself administering an annual Parliamentary Grant-in-aid of £36,000 and a staff of 29. Some measure of the growth of his responsibilities (whilst he was not in the least an "empire builder") may be gained from the fact that the corresponding figures at his death were about £2,000,000 and 100. Following the Scientific Information Conference in 1948, which he organised between scien-

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tists of the Commonwealth and the U.S.A., he persuaded the Royal Society in the 1950s to become its own publisher, and he encouraged other learned societies to do likewise, a step which proved substantially to their benefit. In the years before 1957 he contributed greatly to the organisation of the International Geophysical Year, in which not only were Soviet scientists brought into co-operation, but the Antarctic Treaty was signed, recognising Antarctica as a scientific laboratory above military or commercial interests.

The years after 1957 were charged with the publication of the results of the International Geophysical Year, and with preparations for the celebration of the Society's Tercentenary in 1960. This started with a formal opening by Her Majesty The Queen before an audience of some four thousand in the Albert Hall on 19 July, which was followed by ten days of lectures and visits, including a Service with a congregation of fifteen hundred in St. Paul's Cathedral. The celebrations were a major national event, with many overseas guests who had to be looked after in a manner matching the occasion, and their success was largely due to the efforts of Martin and his relatively small staff.

For some years it had been evident that the activities of the Society were out-growing its premises in Burlington House, and the possibilities of a move

were contemplated. There had been talk of a national science centre somewhere in London where all scientific societies could be brought together, but the proposal that eventually took shape was the move of the Royal Society into new premises in Carlton House Terrace. This was very largely achieved under the Presidency of Lord Florey by Martin, the move finally taking place in 1967 under the Presidency of Professor (later Lord) Blackett. In this way the Society was enabled to stay in most fitting premises in central London; and although these could not become the nucleus of a national science centre, the generous policy of the Society in making its accommodation available to other learned societies for symposia and conferences has helped to make it even more a forum of general scientific activity than it was previously.

The large post-war growth in the Society's activities coincided with Martin's appointment. It was primarily due to the greater national appreciation of the value of science and technology resulting from their effects in World War II. As the time of his appointment the number of Fellows elected annually had just been raised from 20 to 25, and it was increased to 30 in 1965 mainly to give increased recognition to engineering and technology, and to 40 in 1975, partly to recognise the growth of science and technology in the Commonwealth. Martin made a characteristic contribution in effecting all aspects of the expansion so smoothly that very few growing pains were encountered, and the staff that he recruited to support him have unfailingly reflected the courtesy, helpfulness and discretion of which he was a model.

In addition to the obvious landmarks in the evolution of the Royal Society in the past thirty years there have been many other new activities for which he provided enthusiastic support. Relations have been developed and maintained with a wide range of national academies overseas, including those in China, the Commonwealth, Japan, Latin America, the Middle East, the U.S.A., and the U.S.S.R. The International Council of Scientific Unions, too, owes much to Martin's support, as does the European Scientific Exchange Scheme, in which some 90 British scientists are exchanged annually with a corresponding number from the Continent; and in the 1960s he did much to save Aldabra and promote the foundation of the Royal Society re-

search station there. These great peaks of administration, however, convey little idea of the continuous demands made of him in running the many activities of the Society. It has some 120 committees and he was Secretary of nearly all of them. He also attended many of the scientific lectures, for he always retained his interest in science for its own sake.

Ever since Babbage's celebrated attack on the Society in 1830 in his *Reflections on the Decline of Science in England*, the function of the Society has from time to time been questioned. I can remember Martin's chuckle when I drew his attention to the fact that his 1830 predecessor had come directly under Babbage's attack for taking a 10% commission from the keeper of the tavern in which he persuaded the Royal Society to hold its annual dinner. Martin's own quiet good humour contributed to that sense of proportion which he always maintained, both in dealing with the occasional challenges to the Society and with the idiosyncracies of some of its Fellows.

One result of Babbage's attack, incidentally, was that the British Association was formed in order to become the main forum for professional men of science, in contrast to the Society, to which laymen were often elected at that time. Over the years, the functions of the two bodies have tended to exchange, and one of Martin's further interests lay in the Association; he was a member of its Council almost continuously from 1959 onwards. Once again, he played a part in getting Government support for the British

Association, with funds that have been channelled through the Society.

His abilities were very widely drawn upon. He was a member of the Councils of the Royal Society of Arts, the Society for the Protection of Science and Learning, and the Charities Aid Foundation; and he was on the Executive Council of the CIBA Foundation. The Royal Society of Edinburgh elected him a Fellow in 1956. Since 1967 he had been Chairman of the B.B.C. Science Consultative Group, and since 1950 a Commissioner of Income Tax. His work was recognised by the award of a C.B.E. in 1960, and a Knighthood in 1970, and Honorary Degrees of D.Sc. from the University of Edinburgh and of D.C.L. from Newcastle.

But with all his public service, the Royal Society—both for itself and what he believed it could do—was his absorbing interest. It recognised the increased responsibilities of his office when it re-designated him Executive Secretary in 1962. By a Statute of 1847 he could not be a Fellow, for in a sense his post is one in which the holder has to be a man apart from any of the sectional interests that constitute the full spectrum of science: "A means for the Fellows to express their policies" as he himself put it, adding "I have tried to organise affairs so that all the decisions on scientific matters are taken by the Fellows." To him, his part lay in securing all the information that they needed before taking a decision, and in smoothly implementing the decision once it was taken. He was careful not to volunteer guidance but members of Council, and

many of its committee chairmen, gratefully found that he always had something helpful to say when he was asked for his opinion.

Although he could not be a Fellow, few Fellows have done more or had greater influence for good in the Society since World War II than David Martin. With his fervent belief in its cause, his valedictory words are a message for the future: "As the years have passed I have become more and more convinced that it is not enough to leave the advancement of science to scientists alone. The need to see it as making a contribution, and only one contribution, to the world's problems has caused me to recognise the desirability of seeking liaison with other bodies. . . . These bodies operate in different or wider canvasses and, in my belief, operate better in liaison with the scientific activities which the Royal Society represents. The ivory tower concept for scientists is now anachronistic and although I feel an elitist organisation for science has a vital and important role, the base of the pyramid of excellence must reside in the ordinary everyday life of the people."

As for a personal appreciation, what he said of Harold Hartley on the latter's 90th birthday might well have been said of himself: "I would not dissent from a eulogy that ascribed to him the philosophical powers of a Socrates, the strength of a Hercules and the decisive action of a Churchill, but above all he has a warm humanity, which secures a place of affection in the hearts of all those privileged to know him as a friend."

R. V. Jones

Leopold Ruzicka

LEOPOLD RUZICKA, one of the greatest and most influential organic chemists, died on September 26, 1976. His work exemplifies that combination of severely disciplined empiricism with intellectual fantasy and imagination, which is the hallmark of first class organic chemistry. Ruzicka moreover was a unique character with a personality which was perpetually interesting and stimulating to all who knew him. His life also could teach us a lesson sorely needed in the United Kingdom—how to make an effective combination of academic and industrial efforts in research—if only those in authority would pay heed.

Ruzicka was born on September 13, 1887, in Vukovar, a small town in Eastern Croatia where the river Drava joins the Danube. The family name is Czech, going back to his great-grandfather. Ruzicka's father, a cooper and timber merchant, died when the boy was four years old, and the family moved to the neighbouring town of Osijek,

where the young Leopold attended the classical high school, learning much Latin and Greek but neither chemistry nor geometry. At an early age Ruzicka was determined to become a priest, but this ambition had to be abandoned and, after much thought, he decided that his life's work should be in the organic chemistry of natural products. The circumstances which influenced this decision, remarkable at such an early age, are obscure; but we have Ruzicka's own word for it.

At the age of 18 Ruzicka left the Austro-Hungarian domain and sought training in Switzerland or Germany. His first aim was to enter the Polytechnic in Zürich, but the entrance examination was forbidding in its requirement for geometry as well as chemistry. So Leopold went to the Technische Hochschule at Karlsruhe, where he was accepted without examinations. The choice was fortunate because he was free to select the lectures suited to his special interests, and he joined Hermann Staudinger. It

is typical of Ruzicka's extraordinary energy and intellectual ability that in only four years he completed the course for both first and higher degrees, graduating in 1910 as Doctor Ingenieur. Staudinger invited his outstanding pupil to join him in investigating the then unknown constituents, poisonous to insects, of *Chrysanthemum cinerarii-folium*, the pyrethrins now of such economic importance. And in 1912 Staudinger succeeded Richard Willstätter at the Eidgenössische Technische Hochschule, Zürich. So Ruzicka arrived eventually, without examination, at the institute which he was to serve so well for many years. In 1916 he ceased collaboration with Staudinger in order to work on his habilitation thesis, which in 1918 brought him the rank (unpaid) of Privat Dozent and in 1923 of titular Professor. His researches were in the field of alicyclic compounds, which always remained his chemical interest.

Finance was a great problem to the unpaid lecturer, and in 1921 he accepted an invitation to collaborate

with the perfume factory in Geneva, then Naef et Cie and nowadays Firmenich et Cie. So began an academic-industrial collaboration, fruitful for both partners. Ruzicka's research proposal was 'synthesis of the sesquiterpene perfumes farnesol and nerolidol; clarification of the structure of the four important odorous ketones, civetone, muscone, irone, and jasmone'. Until 1925 the work was carried out in Zürich in a cellar, with eventually eight co-workers, but then it was transferred to better quarters in Geneva. It was one of the classical, seminal investigations in organic chemistry. Nowadays it is difficult to remember that accepted dogma precluded stability for macrocyclic carbon structures, such as were proved to be the basis of the commercially important perfumes of musk and civet. The structures of the natural products were elucidated, they were synthesised, and a simpler, cheaper synthetic musk perfume, 'exaltone', was produced. Ruzicka spent only 18 months in the industrial laboratory, but that cemented a lasting relationship between it and the ETH, Zürich.

In 1926 Ruzicka accepted a call to the Professorship in Utrecht, where he pursued the chemistry of the many membered rings and of the higher ketones. In addition he began to acquire that deep love and knowledge of Dutch painting, which was to become almost as important as his love for chemistry. The stay was, however, short because Staudinger's successor at the ETH, Richard Kuhn, returned to Germany, and the President of the ETH, Rohn, came to Utrecht to offer the post to Ruzicka. Thus Ruzicka, who had become a Swiss citizen in 1917, became the first non-German to occupy the chair, although in due course he continued their tradition, still maintained by his own successor, of becoming a Nobel Laureate.

At the ETH Ruzicka threw himself into the task of making it the leading organic chemical institute in the whole world. He built so well that this position has, in the perhaps prejudiced view of the writer, never been lost. Ruzicka had an oft-quoted saying, "academic freedom consists in being allowed to work far harder than is prescribed." He certainly followed his own precept—and made similar demands on his colleagues. From the Zürich laboratory poured many publications opening up the detailed chemistry of the higher terpenes, the steroids and especially the sex hormones. The series of papers *Ueber Steroide und Sexualhormone*, continued by former collaborators, has reached part 256 and another series *Ueber Triterpene* likewise reached part 196. Ruzicka took pride in the way in which these marathons were continued by relays of his pupils. The triterpene

series, notable for the skill and exactitude shown in unravelling immensely difficult problems, was joint work with O. Jeger. Jeger went on to investigate the photochemistry of alicyclic compounds, and yet another long series of papers emerged and yet another first-class chemist, a "chemical grandson" of Ruzicka, K. Schaffner, now at the Max-Planck Institute, Mülheim.

Ruzicka had an instinct for the significance of apparently minor natural products. Few could have discerned that the constituents of wool fat, especially lanosterol, would turn out to have great importance as the precursor of the steroids.

In this work Ruzicka shrewdly enlisted financial support from both the CIBA company in Basle and the Rockefeller Foundation. The close contact with CIBA included supply of personnel for their laboratories and assignment of patents, which proved to be profitable. It is worth noting in passing that Ruzicka's successor, Vladimir Prelog, became a member of the board of CIBA, now CIBA-GEIGY.

While much of these researches comprised detailed experimental exploration of complex organic structures, the organic chemist's characteristic underlying interest in correlating experimental data was never neglected. The isoprenoid origin—in modern terms derived from mevalonate—of these natural products was always in Ruzicka's mind and eventually there emerged the biogenetic isoprene rule. Such interests naturally led Ruzicka towards biochemistry proper, and he was largely instrumental in the establishment of a biochemical institute in the ETH. Moreover his younger colleagues, notably V. Prelog and D. Arigoni, have extended the scope of organic chemical researches into enzymology and experimental, as distinct from merely speculative biosynthesis.

In his prime Ruzicka had a rather conservative view of organic chemical theory, but again it is noteworthy that another of his brilliant pupils, A. Eschenmoser, has transformed much of our outlook on organic chemical synthesis by thorough understanding of theoretical concepts.

Initially Ruzicka had a critical view of physical chemistry, but, as his interest in spectroscopic and other physical methods grew—and he ensured that his institute lacked nothing in instrumentation in that regard—his sympathy for physical chemistry increased. He helped the growth of young physical chemists and the development of a separate institute in the ETH.

The patents on partial synthesis of the male hormones, testosterone and methyltestosterone, generated substantial sums of money. Ruzicka used them to build up a superb collection of Dutch

painting—works by Franz Hals, Rembrandt, Rubens, and many others—which was presented to the Zürich Kunsthau. One example of the quality of this collection will suffice: a Dutch art expert considered that of all the views of Haarlem painted by Jacob van Ruysdael, the finest was in Ruzicka's collection. This collection was not assembled by merely following the advice of art dealers. On the contrary, Ruzicka was a very knowledgeable amateur, in the best sense of the word, and his Zürich endowment includes an important library. The former director of the Mauritshuis in the Hague has testified to Ruzicka's expertise and his characteristic cunning and energy in acquisition of the best pictures for his collection. The writer remembers Ruzicka's indignation when difficulties were raised about an export licence for the portrait *Philip the Fourth* (Rubens) bought in London. Various letters, one translated into "too polite Cambridge English", another in Ruzicka's own words, were ineffective. Eventually a successful arrangement was made through the diplomacy of the Swiss Ambassador in London.

No memorial to Leopold Ruzicka would be complete without reference to his wit and penchant for entertaining stories. Although a very loyal Swiss citizen, he loved to parade his left wing views, suitably exaggerated for the benefit of the conservative Swiss, while being proud of his membership of the Papal Academy of Sciences, not forgetting his simultaneous membership of the Soviet Academy. It is recorded that in a Leningrad hotel he announced loudly that "as we are all good Party members, we can speak freely". As his hosts blanched, he went on to compare unfavourably the contributions made by Stalin to the Hermitage collection with those derived from Catherine the Great. This outspokenness was evident in the laboratory. The writer well remembers a distinguished American author of a popular textbook on organic chemistry, being distinctly disconcerted by the interruption of his lecture in Zürich because Ruzicka would not tolerate a pentavalent carbon atom on the blackboard of his laboratory. But there was no malice in Leopold Ruzicka. He loved to see his younger colleagues develop and the superb institute at Zürich stands as a lasting memorial to his life and work.

Ruzicka detested Nazism, and he gave generous succour to victims of Nazi oppression.

He received, of course, numerous honours, among them the Nobel Prize (1939) and Foreign Membership of the Royal Society (1942).

The autobiographical memoir *In the borderland between bioorganic chemistry and biochemistry*¹ makes rewarding

reading, as does Ruzicka's own obituary notice of Arthur Stoll², which reveals much of Ruzicka himself as well as the subject of the memoir.

The writer thanks Professor O. Jeger and V. Prelog for their help in providing information and advice.

G. W. Kenner

¹ *A. Rev. Biochem.* **43**, 1–20 (1973).

² *Biogr. Mem. Fellows R. Soc.* **18**, 567–593 (1972).

R. A. Morton

RICHARD ALAN MORTON, Emeritus Professor of Biochemistry in The University of Liverpool, an outstanding British biochemist of his generation, died peacefully at his home on January 21 after a short illness; he was seventy-seven. Born in Liverpool of Welsh parents, Professor Morton received his early education at Oulton School. After a short time in the Army he entered Liverpool University as an undergraduate in 1919 and, apart from a sabbatical year in the United States as Visiting Professor at Ohio State University in 1931, remained there until he retired in 1966. He graduated in chemistry and worked for his Ph.D. under Professor E. C. C. Baly, a pioneer in the application of spectroscopy to chemical problems; in 1924 he was appointed special lecturer in spectroscopy in the Chemistry Department where he remained until 1944, when, with great foresight, the University elected him Johnston Professor of Biochemistry. He held this chair, the first established chair of biochemistry in the UK, with great distinction for twenty-two years.

Professor Morton's early research work was concerned with relating the absorption spectra of chemical compounds to their structure and earned him the Royal Institute of Chemistry's Meldola Medal in 1930; later he pioneered the application of absorption spectroscopy to biology. This interest was first aroused in 1926 when he collaborated with Professor (later Sir Ian) Heilbron to show that an impurity in cholesterol isolated from cod liver oil and which exhibited absorption bands in the ultra violet region of the spectrum, was the compound which was converted by ultra violet irradiation into the anti-rachitic principle. These were the days before the structure of cholesterol was known, but the active compound eventually turned out to be 7-dehydrocholesterol. He then particularly concerned himself with vitamin A which had two spectroscopic 'labels', an ultra violet absorption band ($\lambda_{\max}$ 328nm) and the blue colour ($\lambda_{\max}$ 617nm) which it gave on treatment in chloroform with a saturated chloroformic solution of antimony trichloride. Critical examination of the colour test led to his discovery of

vitamin A₂ which was found particularly in liver oils from fresh water fish. Again, thoughtful assessment of the absorption spectrum of 'retinene', the prosthetic group of the visual pigment rhodopsin, began work which led to his discovery in 1941–1942 that retinene was vitamin A aldehyde (retinal) and that the corresponding compound from porphyropsin from fresh water fish retinas was the aldehyde of vitamin A₂. These penetrating discoveries, which gave studies on the biochemistry of visual pigments a new impetus, resulted in Morton's election to the Fellowship of the Royal Society in 1950.

Other important investigations on vitamin A followed but the later stages of Professor Morton's research career saw two extremely important additional discoveries: the first was ubiquinone, discovered independently at the same time in the United States, and the second was the family of polyprenols. The impact of these discoveries on modern biochemistry was profound. The experts on electron transport could from then on carry out their experiments secure in the knowledge that in the case of ubiquinone they were dealing with a well characterised compound. Later work revealed the importance of polyprenols as carrier compounds particularly in cell wall biosynthesis. It gave Professor Morton great satisfaction in his retirement to follow the detailed elucidation of the biochemical significance of these compounds particularly when these developments were carried out by his old students.

During the second world war Morton was concerned with an investigation organised by The Accessory Food Factors Committee of the MRC to determine the vitamin A requirements of humans. The experiments were carried out on conscientious objectors who volunteered as experimental subjects. This stimulated a life-long interest in nutrition and although he never experimented further in nutrition he read deeply in the subject and made many thoughtful contributions to a number of nutritional working parties. In 1969 he was elected a member of The American Institute of Nutrition.

Under Professor Morton's sympathetic guidance the Department of Biochemistry at Liverpool prospered and his crowning achievement was to persuade the University to build the large new building in which the department is now housed. He developed an effective research school and a remarkably large number of his students now hold positions of high responsibility in places as far apart as India and Canada. Professor Morton's great gifts as a scientist and as a wise counsellor became well known outside the University early in his career and he was constantly in

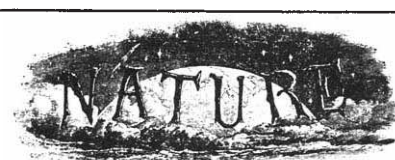
demand for professional committees; he served twice on the Council of the Royal Society, was Chairman of the Biochemical Society and was a member of many government committees. From 1963 to 1968, he held the important position of Chairman of the Committee on Food Additives. He enjoyed writing and editing and was a member of the Editorial Board of the Biochemical Journal for seven years and Chairman of the Publications Board of the Royal Society 1961–1962.

He received honorary degrees from the University of Wales, Trinity College Dublin and The University of Coimbra, where his first research student became Rector. When he retired he was elected an Honorary Member of the Biochemical Society.

In retirement he continued to be as active as ever and he retained his fresh and enthusiastic approach to biochemistry right to the end. Indeed he had only just returned from an international symposium in India and at the time of his death was busy editing the proceedings. He spent a great deal of time on the work of the Oceanography and Fisheries Committee of the Natural Environment Research Council and in 1969 was Royal Society Visiting Professor at The Royal University of Malta. In spite of all this extramural activity he found time to complete two projects close to his heart: one was the *History of the Biochemical Society* (1911–1969) and the other a mammoth two volume new edition of his book *Absorption spectra of Vitamins and Hormones* last published in 1942.

In spite of his great achievements and international renown Professor Morton remained unassuming, friendly and approachable. His life shows that shrewdness and devotion to the demanding career of a scientist need not be divorced from kindness, gentleness and sympathy. His many friends and colleagues will remember Alan Morton as a distinguished scientist, wise in his judgement, humane in his outlook, whose long career brought lustre to biochemistry and to the University of Liverpool.

T. W. Goodwin



A hundred years ago

A bright violet meteor was observed at St Etienne on 11 March at two o'clock in the morning, in the southern part of the horizon. It was travelling with great velocity from west to east. No detonation was heard.

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